

# The acute effects of 5-MeO-DMT on behaviour, brain activity and arousal in mice



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## Personal acknowledgements

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## **Declaration of contributions**

I declare that no part of this thesis has been or is being submitted for any qualification other than the degree of Doctor of Philosophy at the University of Oxford.

The work presented here is original and has been carried out under the supervision of Prof. Vladyslav Vyazovskiy (Department of Physiology, Anatomy and Genetics), Prof. David Bannerman (Department of Experimental Psychology), and Prof. Trevor Sharp (Department of Pharmacology).

Dr McKillop trained me at animal handling and injections. Dr Blanco-Duque, Dr Krone, Dr McKillop, Dr Prius-Mengual, Dr Wilcox and Dr Chakrabarty trained me for the surgeries. Dr Prius-Mengual conducted the intra-cortical implants of Chapter 3. The design of the oculometre was based on a light-stimulation LED device made by Dr Prius-Mengual. The drawing of the mice wearing an oculometre in Chapter 4 was made by Vanda Reiss. Methods to utilise Deep Lab Cut were developed jointly with Jasmin Patel.



# Abstract

The serotonergic system plays an important role in the subcortical control of global sleep-wake states, as supported by observations that the administration of serotonergic psychedelics suppresses paradoxical sleep and results in increased sleep fragmentation. Growing evidence also suggests that a single psychedelic experience can positively impact long-term psychological well-being, with relevance to the treatment of psychiatric disorders often associated with disturbed sleep. Yet, little is known about how psychedelics affect sleep and wake states, and the possibility that the administration of psychedelics results in the occurrence of an altered state of vigilance has received less attention. The aim of this DPhil project is to characterise the acute effects of psychedelics on sleep-wake states, behaviour, brain activity, sensory responsiveness, and arousal in laboratory mice.

This DPhil thesis is divided into four results chapters. First, I present results showing that an injection of 5-MeO-DMT acutely disrupt vigilance states by inducing sleep-like patterns of brain activity in awake and behaving adult male mice. The next part focuses on developing a novel approach to measuring the levels of arousal by recording videos of the pupil diameter in freely behaving mice. In this part I demonstrate that pupil mydriasis induced by 5-MeO DMT correlates with the occurrence of a dissociated brain state. The following part addresses the effects of 5-MeO DMT on EEG responses evoked by auditory stimulations. Here I observe characteristic changes in evoked potentials during the acute state following an injection of 5-MeO-DMT, reflected in an elongation in the otherwise stereotypical elements of the evoked responses. In the final part, I explore the potential regulatory mechanisms underlying the 5-MeO-DMT-induced dissociated state. Here I demonstrate that slow waves induced by psychedelics are unlikely to be homeostatically regulated, and, furthermore, local injections of the compound in the neocortex do not trigger local changes in cortical activity. Finally, I show that administration of an antagonist of 5-HT<sub>1A</sub> receptors partially restores 5-MeO DMT induced changes in sleep architecture, but seems to exacerbate its effects on behaviour.

Taken together, my results suggest that psychedelics such as 5-MeO-DMT induce a dissociated state of vigilance, altering levels of arousal and responses to external

stimulation during wakefulness. This supports the notion that the serotonergic system is involved in the control of qualitative aspects of vigilance states such as brain activity patterns and levels of arousal. Furthermore, these results offer a novel insight into the global effects of psychedelics on brain activity. This in turn provides mechanistic insights into the putative therapeutic effects of psychedelics.

Further investigations of the effects of psychedelics on sleep and wakefulness could lead to a better understanding of their neurophysiology as well as local and global regulation. Integrating research on sleep and psychedelic can offer new perspective on both topics and improve the comprehension of brain states.

# Contents

|                                                                          |             |
|--------------------------------------------------------------------------|-------------|
| <b>List of Figures</b>                                                   | <b>ix</b>   |
| <b>List of Tables</b>                                                    | <b>xi</b>   |
| <b>List of Abbreviations</b>                                             | <b>xiii</b> |
| <b>1 General introduction</b>                                            | <b>1</b>    |
| 1.1 Concerning psychedelics . . . . .                                    | 1           |
| 1.2 The study of vigilance states . . . . .                              | 13          |
| 1.3 Regulation and functions of sleep . . . . .                          | 22          |
| 1.4 Restoring mental health . . . . .                                    | 37          |
| 1.5 Hypothesis and aims . . . . .                                        | 42          |
| <b>2 General methods</b>                                                 | <b>45</b>   |
| 2.1 Animal husbandry . . . . .                                           | 46          |
| 2.2 Surgeries . . . . .                                                  | 47          |
| 2.3 Injections . . . . .                                                 | 52          |
| 2.4 Sleep deprivation . . . . .                                          | 53          |
| 2.5 Data acquisition and processing . . . . .                            | 54          |
| <b>3 The effects of 5-MeO-DMT on vigilance states and brain activity</b> | <b>59</b>   |
| 3.1 Introduction . . . . .                                               | 60          |
| 3.2 Methods . . . . .                                                    | 66          |
| 3.3 Results . . . . .                                                    | 70          |
| 3.4 Discussion . . . . .                                                 | 83          |
| <b>4 The development of new methods to measure attention and arousal</b> | <b>91</b>   |
| 4.1 Introduction . . . . .                                               | 92          |
| 4.2 Methods . . . . .                                                    | 99          |
| 4.3 Results . . . . .                                                    | 108         |
| 4.4 Discussion . . . . .                                                 | 118         |

|                   |                                                                            |            |
|-------------------|----------------------------------------------------------------------------|------------|
| <b>5</b>          | <b>The effects of 5-MeO-DMT on auditory evoked response potentials</b>     | <b>127</b> |
| 5.1               | Introduction . . . . .                                                     | 128        |
| 5.2               | Methods . . . . .                                                          | 133        |
| 5.3               | Results . . . . .                                                          | 138        |
| 5.4               | Discussion . . . . .                                                       | 148        |
| <b>6</b>          | <b>The potential mechanisms underlying the effects of 5-MeO-DMT</b>        | <b>155</b> |
| 6.1               | Introduction . . . . .                                                     | 156        |
| 6.2               | Methods . . . . .                                                          | 163        |
| 6.3               | Results . . . . .                                                          | 168        |
| 6.4               | Discussion . . . . .                                                       | 183        |
| <b>7</b>          | <b>General discussion</b>                                                  | <b>193</b> |
| 7.1               | Summary . . . . .                                                          | 194        |
| 7.2               | A proposed circuitry of 5-MeO-DMT activation. . . . .                      | 197        |
| 7.3               | Exploring the delay of paradoxical sleep . . . . .                         | 202        |
| 7.4               | Slow waves as a mechanism supporting sleep and therapeutic processes       | 205        |
| 7.5               | A brain-wide state for psychedelic therapeutic action . . . . .            | 209        |
| 7.6               | Conclusion . . . . .                                                       | 211        |
| <b>Appendices</b> |                                                                            |            |
| <b>A</b>          | <b>Analysis tables of Chapter 3</b>                                        | <b>215</b> |
| <b>B</b>          | <b>Analysis tables of Chapter 4</b>                                        | <b>223</b> |
| <b>C</b>          | <b>Supplementary materials of Chapter 5</b>                                | <b>229</b> |
| C.1               | Histology . . . . .                                                        | 229        |
| C.2               | ERP component analysis . . . . .                                           | 230        |
| C.3               | Analysis tables . . . . .                                                  | 235        |
| <b>D</b>          | <b>Supplementary materials of Chapter 6</b>                                | <b>247</b> |
| D.1               | Histology . . . . .                                                        | 247        |
| D.2               | 5-HT <sub>1A</sub> antagonist effects on auditory ERP components . . . . . | 248        |
| D.3               | Analysis tables . . . . .                                                  | 250        |
| <b>References</b> |                                                                            | <b>263</b> |

## List of Figures

|     |                                                                                       |     |
|-----|---------------------------------------------------------------------------------------|-----|
| 1.1 | The chemical structures of psychedelics . . . . .                                     | 4   |
| 1.2 | 5-HT <sub>2A</sub> receptor G-protein signalling pathway in neurons . . . . .         | 6   |
| 1.3 | 5-HT <sub>1A</sub> receptor G-protein signalling pathway in neurons . . . . .         | 7   |
| 1.4 | The early ages of sleep research . . . . .                                            | 14  |
| 1.5 | The discovery of a paradoxical state . . . . .                                        | 16  |
| 1.6 | Projections of the 5-HT system . . . . .                                              | 24  |
| 1.7 | The two-process model of sleep regulation . . . . .                                   | 29  |
| 2.1 | General experimental plan . . . . .                                                   | 48  |
| 3.1 | The electrophysiology of vigilance states . . . . .                                   | 60  |
| 3.2 | Experimental plan of Chapter 3 . . . . .                                              | 67  |
| 3.3 | The analysis of head twitches . . . . .                                               | 71  |
| 3.4 | The effects of 5-MeO-DMT on behaviour . . . . .                                       | 73  |
| 3.5 | Representative effects of 5-MeO-DMT injections on brain activity .                    | 75  |
| 3.6 | The alteration of EEG power by 5-MeO-DMT . . . . .                                    | 76  |
| 3.7 | Intracortical activity in the visual cortex after an injection of 5-MeO-DMT . . . . . | 78  |
| 3.8 | Changes in sleep architecture following 5-MeO-DMT administration                      | 80  |
| 3.9 | Changes in sleep EEG activity following 5-MeO-DMT administration                      | 82  |
| 4.1 | Experimental plan of Chapter 4 . . . . .                                              | 100 |
| 4.2 | Details of the oculometre . . . . .                                                   | 106 |
| 4.3 | Effects of sleep deprivation on attention . . . . .                                   | 109 |
| 4.4 | Pilot observations of the pupil in anaesthetised animals . . . . .                    | 111 |
| 4.5 | Representative visualisation of the oculometre . . . . .                              | 113 |
| 4.6 | Effects of 5-MeO-DMT on pupil diameter alone . . . . .                                | 115 |
| 4.7 | Effects of 5-MeO-DMT on EEG and pupil diameter . . . . .                              | 116 |
| 4.8 | Correlations between EEG activity and pupil diameter . . . . .                        | 117 |
| 5.1 | Comparison of evoked responses between mice and humans . . . . .                      | 129 |
| 5.2 | Experimental plans of Chapter 5 . . . . .                                             | 134 |
| 5.3 | Evoked response potential across vigilance states . . . . .                           | 139 |

|     |                                                                                                                   |     |
|-----|-------------------------------------------------------------------------------------------------------------------|-----|
| 5.4 | Sleep onset and ERP profiles after stimulations in different frequencies                                          | 140 |
| 5.5 | The effects of 5-MeO-DMT on EEG and LFP power spectra . . . .                                                     | 142 |
| 5.6 | The effects of 5-MeO-DMT on ERPs . . . . .                                                                        | 144 |
| 5.7 | Representative intracortical ERPs . . . . .                                                                       | 145 |
| 5.8 | Intracortical ERPs during wake and sleep . . . . .                                                                | 146 |
| 5.9 | The effects of 5-MeO-DMT on intracortical ERPs . . . . .                                                          | 147 |
| 6.1 | The regulation of wake and sleep . . . . .                                                                        | 157 |
| 6.2 | Experimental plans of Chapter 6 . . . . .                                                                         | 163 |
| 6.3 | The effects of sleep deprivation on 5-MeO-DMT-induced changes in<br>EEG activity . . . . .                        | 169 |
| 6.4 | The effects of sleep deprivation on subsequent sleep episodes . . . .                                             | 171 |
| 6.5 | The effects of intracortical injections of 5-MeO-DMT in the SSCx .                                                | 174 |
| 6.6 | The effects of 5-MeO-DMT on EEG and LFP spectrum with a 5-HT <sub>1A</sub><br>receptor antagonist . . . . .       | 177 |
| 6.7 | Time course of EEG powers and pupil dilation in response to a<br>5-HT <sub>1A</sub> antagonist . . . . .          | 179 |
| 6.8 | ERPs in response to 5-MeO-DMT injection and the presence of a<br>5-HT <sub>1A</sub> receptor antagonist . . . . . | 182 |
| 7.1 | A summary of the effects of 5-MeO-DMT on brain activity mediated<br>by 5-HT neurotransmission. . . . .            | 199 |
| C.1 | Histology results of Chapter 5 . . . . .                                                                          | 229 |
| C.2 | EEG ERP components amplitude across wake and sleep . . . . .                                                      | 231 |
| C.3 | EEG ERP components latencies across wake and sleep . . . . .                                                      | 232 |
| C.4 | EEG ERP components amplitude changes between wake and SWS .                                                       | 233 |
| C.5 | EEG ERP components latency changes between wake and SWS . .                                                       | 233 |
| C.6 | The effects of 5-MeO-DMT on EEG ERP components amplitude . .                                                      | 234 |
| C.7 | The effects of 5-MeO-DMT on EEG ERP components latencies . .                                                      | 235 |
| D.1 | Histology results of Chapter 6 . . . . .                                                                          | 247 |
| D.2 | EEG ERP component changes with the presence of a 5-HT <sub>1A</sub> receptor<br>antagonist . . . . .              | 249 |

# List of Tables

|      |                                                             |     |
|------|-------------------------------------------------------------|-----|
| 1.1  | Table of discussed psychedelic compounds . . . . .          | 8   |
| 1.2  | 5-HT receptor subtypes and expression . . . . .             | 26  |
| A.1  | Statistical analyses for figure 3.3 . . . . .               | 215 |
| A.2  | Statistical analyses for figure 3.4 . . . . .               | 216 |
| A.3  | Statistical analyses for figure 3.6.A-B . . . . .           | 217 |
| A.4  | Statistical analyses for figure 3.6.C-E . . . . .           | 218 |
| A.5  | Statistical analyses for figure 3.8.A . . . . .             | 219 |
| A.6  | Statistical analyses for figure 3.8.B . . . . .             | 220 |
| A.7  | Statistical analyses for figure 3.8.C-G . . . . .           | 221 |
| A.8  | Statistical analyses for figure 3.9 . . . . .               | 222 |
| B.1  | Statistical analyses for figure 4.3 . . . . .               | 224 |
| B.2  | Statistical analyses for figure 4.6 . . . . .               | 225 |
| B.3  | Statistical analyses for figure 4.7.A-B . . . . .           | 225 |
| B.4  | Statistical analyses for figure 4.7.C-F . . . . .           | 226 |
| B.5  | Statistical analyses for figure 4.8 . . . . .               | 227 |
| C.1  | Statistical analyses for figure 5.4 . . . . .               | 236 |
| C.2  | Statistical analyses for figure 5.5 . . . . .               | 238 |
| C.3  | Statistical analyses for figure 5.6 . . . . .               | 239 |
| C.4  | Statistical analyses for figure 5.8 . . . . .               | 240 |
| C.5  | Statistical analyses for figure 5.9 . . . . .               | 241 |
| C.6  | Statistical analyses for figure C.2 . . . . .               | 242 |
| C.7  | Statistical analyses for figure C.3 . . . . .               | 243 |
| C.8  | Statistical analyses for figure C.4 . . . . .               | 244 |
| C.9  | Statistical analyses for figure C.5 . . . . .               | 244 |
| C.10 | Statistical analyses for figure C.6 . . . . .               | 245 |
| C.11 | Statistical analyses for figure C.7 . . . . .               | 246 |
| D.1  | Statistical analyses for figure 6.3.A-B & 6.4.A-B . . . . . | 251 |
| D.2  | Statistical analyses for figure 6.3D-F. . . . .             | 253 |
| D.3  | Statistical analyses for figure 6.4.C-E . . . . .           | 254 |

|      |                                                   |     |
|------|---------------------------------------------------|-----|
| D.4  | Statistical analyses for figure 6.5.A . . . . .   | 254 |
| D.5  | Statistical analyses for figure 6.5.B-F . . . . . | 255 |
| D.6  | Statistical analyses for figure 6.5.G-J . . . . . | 256 |
| D.7  | Statistical analyses for figure 6.6 . . . . .     | 257 |
| D.8  | Statistical analyses for figure 6.7.A-D . . . . . | 259 |
| D.9  | Statistical analyses for figure 6.7.E-F . . . . . | 261 |
| D.10 | Statistical analyses for figure 6.8 . . . . .     | 261 |
| D.11 | Statistical analyses for figure D.2 . . . . .     | 262 |



## List of Abbreviations

|                            |           |                                                |
|----------------------------|-----------|------------------------------------------------|
| <b>5-HT</b>                | . . . . . | Serotonin                                      |
| <b>5-CSRT</b>              | . . . .   | 5-choice serial reaction time                  |
| <b>5-MeO-DMT</b>           | . .       | 5-methoxy-N,N-dimethyltryptamine               |
| <b><math>\delta</math></b> | . . . . . | Delta (0.5 - 4 Hz)                             |
| <b><math>\theta</math></b> | . . . . . | Theta (6 - 10 Hz)                              |
| <b>Ach</b>                 | . . . . . | Acetylcholine                                  |
| <b>AP</b>                  | . . . . . | Anteroposterior                                |
| <b>BSB</b>                 | . . . . . | Biomedical Sciences Building                   |
| <b>CBT</b>                 | . . . . . | Cognitive behavioural therapy                  |
| <b>DMT</b>                 | . . . . . | Dimethyltryptamine                             |
| <b>DOI</b>                 | . . . . . | 2,5-Dimethoxy-4-iodoamphetamine                |
| <b>DRN</b>                 | . . . . . | Dorsal raphe nucleus                           |
| <b>DV</b>                  | . . . . . | Dorsoventral                                   |
| <b>EEG</b>                 | . . . . . | Electroencephalogram / Electroencephalographic |
| <b>EMG</b>                 | . . . . . | Electromyogram / Electromyographic             |
| <b>ERP</b>                 | . . . . . | Evoked response potential                      |
| <b>HTR</b>                 | . . . . . | Head twitch response                           |
| <b>IQRS</b>                | . . . . . | Interquartile ranges                           |
| <b>LC</b>                  | . . . . . | Locus Coeruleus                                |
| <b>LFP</b>                 | . . . . . | Local field potentials                         |
| <b>LSD</b>                 | . . . . . | Lysergic acid diethylamide                     |
| <b>MAOI</b>                | . . . . . | Monoamine oxidase inhibitor                    |
| <b>MDMA</b>                | . . . .   | 3,4-methylenedioxymethamphetamine              |
| <b>ME</b>                  | . . . . . | Mixed-effect                                   |
| <b>ML</b>                  | . . . . . | Mediolateral                                   |

|             |                                                      |
|-------------|------------------------------------------------------|
| <b>MUA</b>  | Multiunit analysis                                   |
| <b>N40</b>  | Negative wave, approximately 40 ms post-stimulation  |
| <b>NA</b>   | Noradrenaline (norepinephrine)                       |
| <b>NMDA</b> | N-methyl-D-aspartate                                 |
| <b>NREM</b> | non rapid eye movement                               |
| <b>P120</b> | Positive wave, approximately 120 ms post-stimulation |
| <b>P20</b>  | Positive wave, approximately 20 ms post-stimulation  |
| <b>PS</b>   | Paradoxical sleep                                    |
| <b>PW</b>   | Paradoxical wake                                     |
| <b>REM</b>  | Rapid eye movement                                   |
| <b>RM</b>   | Repeated measures                                    |
| <b>SCN</b>  | Suprachiasmatic nucleus                              |
| <b>SD</b>   | Sleep deprivation                                    |
| <b>SEM</b>  | Standard error to the mean                           |
| <b>SHY</b>  | Synaptic homeostasis hypothesis                      |
| <b>SLD</b>  | Sublaterodorsal nucleus                              |
| <b>SS</b>   | Serotonin syndrome                                   |
| <b>SSRI</b> | Selective serotonin reuptake inhibitor               |
| <b>SWA</b>  | Slow wave activity                                   |
| <b>SWS</b>  | Slow wave sleep                                      |
| <b>TBG</b>  | Tabernanthalog                                       |
| <b>VLPO</b> | Ventrolateral preoptic area                          |
| <b>VTa</b>  | Ventral tegmental area                               |
| <b>WAY</b>  | WAY100635                                            |
| <b>ZT</b>   | Zeitgeber time                                       |

# 1

## General introduction

### Contents

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|            |                                                         |           |
|------------|---------------------------------------------------------|-----------|
| <b>1.1</b> | <b>Concerning psychedelics</b>                          | <b>1</b>  |
| 1.1.1      | An overview of pre-1970 research on psychedelics        | 1         |
| 1.1.2      | The pharmacology of psychedelics                        | 4         |
| 1.1.3      | The therapeutic effects of psychedelics                 | 10        |
| <b>1.2</b> | <b>The study of vigilance states</b>                    | <b>13</b> |
| 1.2.1      | The origin and definition of vigilance states           | 13        |
| 1.2.2      | The current understanding of vigilance states           | 16        |
| 1.2.3      | Slow waves as a hallmark feature of sleep               | 19        |
| <b>1.3</b> | <b>Regulation and functions of sleep</b>                | <b>22</b> |
| 1.3.1      | The circuitry of vigilance states                       | 22        |
| 1.3.2      | The regulation of sleep                                 | 28        |
| 1.3.3      | The hypothesized function of sleep                      | 32        |
| <b>1.4</b> | <b>Restoring mental health</b>                          | <b>37</b> |
| 1.4.1      | The relationship between sleep and mental health        | 37        |
| 1.4.2      | The association of psychedelics and sleep               | 38        |
| 1.4.3      | Vigilance states are an overlooked yet essential notion | 40        |
| <b>1.5</b> | <b>Hypothesis and aims</b>                              | <b>42</b> |

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## 1.1 Concerning psychedelics

### 1.1.1 An overview of pre-1970 research on psychedelics

The meaning and origin of the word “psychedelic” requires a brief introduction. Since the ground-breaking arrival of this class of drug in the global and scientific western

culture, finding a fitting name for it has sparked discussion, as each name proposed reveals the context around which these drugs were studied or used. The first lexical field surrounding psychedelic drugs was medical. They were called *shizogens*, *psychotica*, *psychotogen*, or *psychotomimetics* as a reference to the property of psychedelics to induce a state sharing some similarities, actual or perceived, with dementia and other mental disorders. Indeed, this class of drug capable of *mimicking psychosis* was firstly used in the western world to understand the diseases of the human mind such as schizophrenia (Osmond, 1957). But, as Osmond writes, “this capacity is not their only, nor even, perhaps, their most important quality.”

Before their arrival in the western scientific community, these compounds were known to strongly alter human emotions and behaviours. Transitioning from a medical to a recreational use, they were referred to as hallucinogens, *which induces hallucinations*<sup>1</sup>, as hallucinations were perceived as an arguably chief symptom of these drugs by the public (Carhart-Harris & Goodwin, 2017; Osmond, 1957; Rucker et al., 2018). In order to relieve this class of drugs from any negative meaning and steer it away from the lexicon of pathology, Osmond coined the neutral term psychedelics. Mind-manifesting, or mind revealing, reflecting that these are substances that yes, mimic psychosis, “but they do much more” (Osmond, 1957).

Psychedelics are psychoactive drugs known to alter emotion, perception, and human behaviour (Barsuglia et al., 2018; Carhart-Harris et al., 2016b; Carter et al., 2005; Halberstadt et al., 2020; Pollan, 2019). They were first introduced to the western scientific community when German scientist Arthur Heffter isolated mescaline in 1897, but culturally, the synthesis of 25-lysergic acid diethylamide (LSD) by Hofmann in 1938 and its subsequent ingestion in 1943 caught the western public eye (Hofmann & Feilding, 2013; Rucker et al., 2018). Psychedelics have been in use in other parts of the world earlier than that, as mescaline from peyote cacti

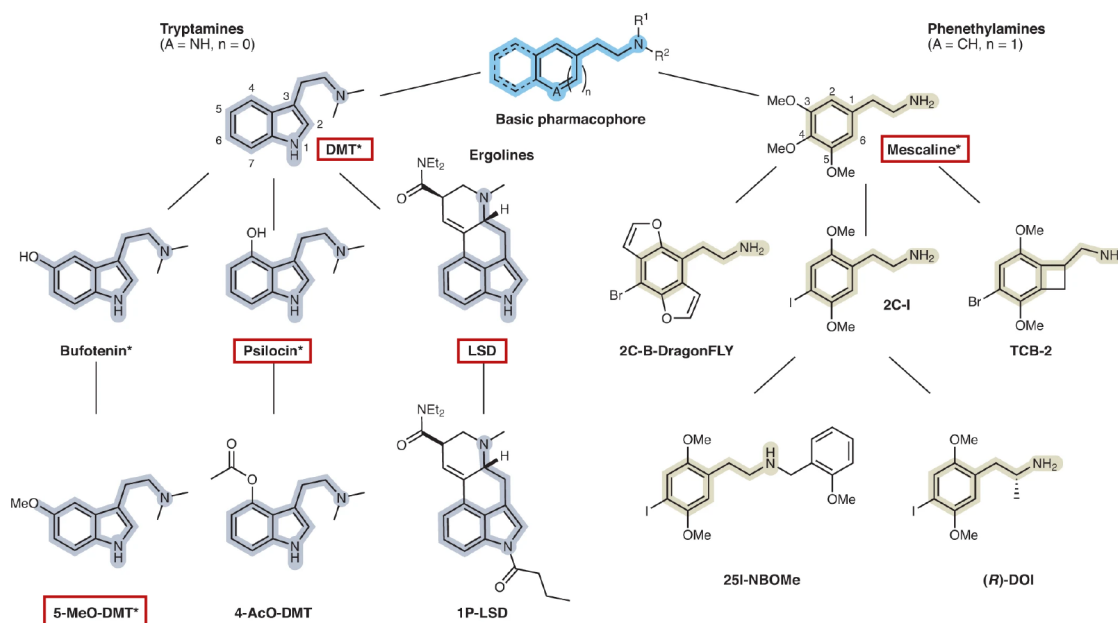
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<sup>1</sup>From the Latin *hallucinatus* (to wander in the mind) and Greek *genesis* (to be born). The definition of hallucinations and their nature can be largely discussed. Sacks writes “Hallucinations are defined as percepts arising in the absence of any external reality – seeing things or hearing things that are not there” (Sacks, 2012).

(*Lophophora williamsii*), “magic” mushrooms and other psychedelic substances such as dimethyltryptamine (DMT)-containing ayahuasca preparations have been commonly used in Native American religious ceremonies (Letcher, 2007), with evidence of mescaline ceremonies dating back to 5700 BCE (Bruhn et al., 2002).

Before the 1970s, psychedelic research investigated the effects of psychedelic drugs on several psychotic disorders due to the similar symptoms they would induce (Rucker et al., 2018). Studies with LSD and mescaline reported either acute aggravation of schizophrenic symptoms (Hoch et al., 1952; Liddell & Weil-Malherbe, 1953; Pennes, 1954), or mixed results between complete remission and worsening of symptoms (Denber & Merlis, 1955; Merlis, 1957). Psychedelics were also used as a tool to assist psychotherapy, as due to the sense of ego-dissolution was thought to resemble hypnosis. At the time most of the studies were deemed successful despite either lacking a control group, relying on subjective results, and lacking any form of meaningful statistical analysis, as reviewed in Rucker et al. (2018) (Chandler, 1960; Sandison & Whitelaw, 1957; Sandison et al., 1954; Whitaker, 1964a, 1964b). The 1960s were marked by the emergence of a counterculture, and the recreational use of psychedelics boomed in the western world, as well as associated safety concerns. With the increasing incidence of studies with psychedelics of questionable ethics by academic and military institutions across Europe and the Americas sparking public outrage (J. K. Larsen, 2016; M. A. Lee & Shlain, 2007; Rucker et al., 2018), the 1967 UN Convention on Drugs upon the resolution WHA20.42 of the Twentieth World Health Assembly urged governments to take immediate action against psychedelics (resolution 1197(XLII)) thus restricting their use for scientific research, followed by further stringent actions in 1968 (resolution 1294(XLIV)) (UN. Economic and Social Council (42nd sess.), 1967; UN. Economic and Social Council (44th sess.), 1968; World Health Organisation, 1967).

These international resolutions put a strong halt on psychedelic research. However, several reviews agree that three key papers in the 1990s allowed what is now considered a revival of psychedelics as a field of research (Carhart-Harris & Goodwin, 2017; Rucker et al., 2018). On healthy subjects, administration of DMT



**Figure 1.1: The chemical structures of psychedelics.** Adapted from Kwan et al. (2022): “The basic psychedelic pharmacophore is highlighted in blue. Tryptamine and phenethylamine pharmacophores are highlighted in grey and yellow, respectively. Ergolines (LSD and 1-propionyl-d-LSD (1P-LSD)) can be viewed chemically as a specialized case of tryptamines. Branches indicate structurally related compounds. Natural products are indicated with asterisks. 4-AcO-DMT: 4-acetoxy-DMT.” Boxed in red are the compound commonly discussed in Chapter 1

(Strassman & Qualls, 1994), psilocybin (Vollenweider et al., 1997) and mescaline (Hermle et al., 1998) were performed to characterise the acute physiological effects of psychedelics on humans and correlate them to psychosis symptoms, leading to a better understanding of the neuropharmacological mechanisms underlying the acute and potential long-term effects of psychedelics.

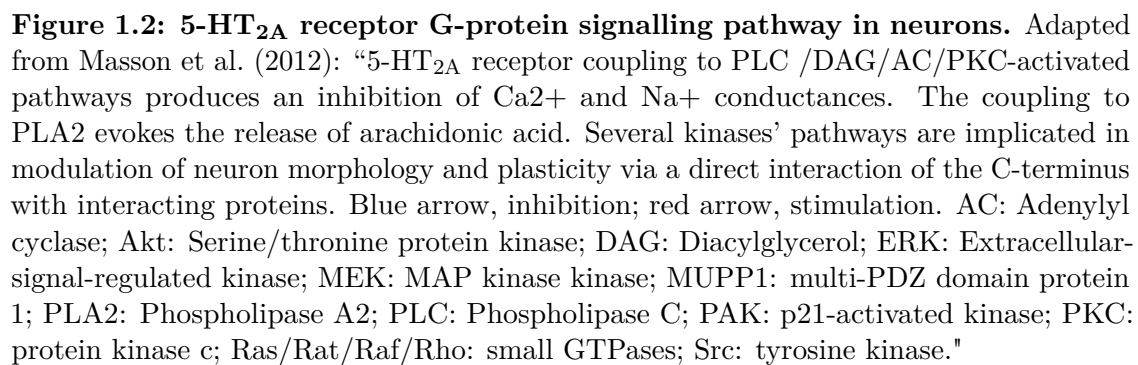
### 1.1.2 The pharmacology of psychedelics

The term “psychedelic drug” encompasses quite a large variety of compounds naturally present in plants, animals, and fungi: LSD was first synthesised from ergot fungi (Hofmann & Feilding, 2013); psilocybin can be extracted from “magic” mushrooms of the *Psilocybe* genus, amongst other genera; mescaline has been isolated from a button of peyote cactus; 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) is secreted by the Sonoran desert toad *Incilius alvarius*. According to

human reports and questionnaires, all of these drugs have psychoactive effects on perception leading to synaesthesia, an increased sense of mysticism, ego dissolution, and dream-like experiences (Barrett et al., 2015; Barsuglia et al., 2018; Carter et al., 2005; Halberstadt et al., 2020). These effects are commonly known as the “psychedelic experience” and they allow a first distinction between psychedelic drugs and other hallucinogens (Halberstadt & Geyer, 2010). At a molecular level, the structure of psychedelics allows for a clarification as well. Psychedelics such as psilocybin and its active form 4-hydroxy-N,N-dimethyltryptamine (psilocin), 5-MeO-DMT and LSD are substituted tryptamines and thus have an indole core analogous to the serotonin (5-HT) neurotransmitter (Figure 1.1). Mescaline is a phenethylamine which, despite the lack of indole core shares the same properties as the aforementioned compounds (Kwan et al., 2022) (Figure 1.1).

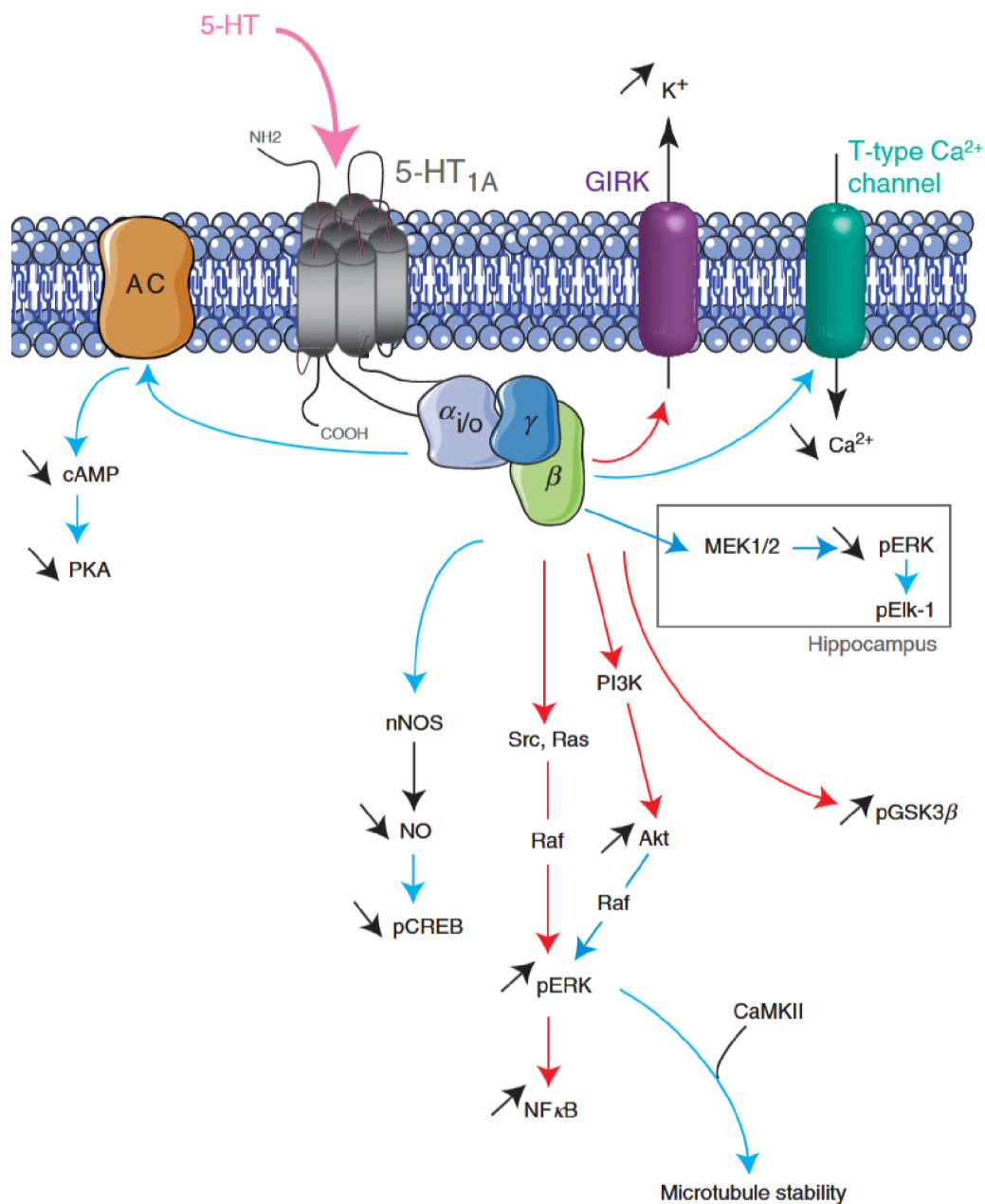
The pharmacology underlying the psychedelic effects can be used to refine the definition of psychedelics further. Indeed, in a recent publication, Nichols, Nichols and Hendricks propose a consensus on only calling psychedelic 5-HT analogous substance with an elevated affinity for 5-HT class receptors and more specifically the 5-HT<sub>2A</sub> receptors (Nichols et al., 2022). This, however, is criticised as numerous non-serotonergic receptor agonists previously labelled as psychedelics might lose some scientific interest despite sharing similar psychoactive and therapeutic effects as LSD, psilocybin and mescaline. This is the case of the indirect 5-HT agonist 3,4-methylenedioxymethamphetamine (MDMA) or the anaesthetic ketamine (Lepow et al., 2023; O’Donnell et al., 2023; Zanos et al., 2018). In this thesis, I will consider psychedelics as a wider group based on the effects of the drugs, with the terminology *classical* psychedelics used to refer to 5-HT analogous compounds.

*Classical* psychedelics are non-selective 5-HT receptor agonists with a highest affinity for the 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> receptors as well as other 5-HT receptors, as detailed in Table 1.1 (Nichols et al., 2022; O’Donnell et al., 2023). Both receptors are widely present in the cortex and subcortical nuclei (Nichols & Nichols, 2008; Sharp & Barnes, 2020). They are G-protein coupled receptors and, when activated, trigger



numerous downstream effects are illustrated in Figures 1.2 & 1.3 5-HT<sub>2A</sub> receptor occupancy has been shown to be associated with the psychotropic effects in humans as 5-HT<sub>2A</sub> receptor antagonists ketanserin and risperidone blocked these effects (Madsen et al., 2019; Vollenweider et al., 1998). The 5-HT<sub>2A</sub> receptors are highly expressed in the cortex of rats, more specifically in pyramidal cells of layers II/III and layers V/VI at the level of the apical dendrites (Cornea-Hébert et al., 1999), but also in the amygdala and the hippocampus of rats and mice (Sharp & Barnes, 2020). It has also been reported that 5-HT<sub>2A</sub> receptors are expressed on large- and





**Figure 1.3: 5-HT<sub>1A</sub> receptor G-protein signalling pathway in neurons.** Adapted from Masson et al. (2012): "The 5-HT<sub>1A</sub> receptor is negatively coupled to AC/PKA/cAMP signaling via Gα<sub>i/o</sub> proteins. Its activation decreases neurotransmitter release in neurons through opposite changes in K<sup>+</sup> (increase) and Ca<sup>2+</sup> (decrease) conductances. 5-HT<sub>1A</sub> receptor stimulation also regulates ERK phosphorylation. Blue arrow, inhibition; red arrow, stimulation. AC: Adenylyl cyclase; Akt: Serine/threonine protein kinase; CaMKII: Ca<sup>2+</sup>/calmodulin-dependent protein kinase II; cAMP: Cyclic adenosine monophosphate; CREB: cAMP response element-binding; GSK3: Glycogen synthase kinase-3; Elk-1: Transcription factor of E twenty six-like family; ERK: Extracellular-signal-regulated kinase; NFκB: Nuclear factor k-light-chain-enhancer of activated B cells; NOS: Nitric oxide synthase; PKA: Protein kinase A; Ras/Rat/Raf/Rho: small GTPases."

**Table 1.1: Table of discussed psychedelic compounds.** Adapted from Halberstadt and Geyer (2011) and Halberstadt (2015). A bold receptor type indicates a markedly higher affinity for said receptor according to the literature.

| Family            | Compound                    | ranked 5-HT receptor affinity (agonism)                                                                                                                                                                                   | Other targets                                                                                                                                                       |
|-------------------|-----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Tryptamines       | <b>5-MeO-DMT</b>            | <b>5-HT<sub>1A</sub></b> ,<br>5-HT <sub>2A</sub> , 5-HT <sub>2C</sub> ,<br>5-HT <sub>7</sub>                                                                                                                              | Substrate for 5-HT transporter,<br>minor affinity for dopaminergic (D <sub>1-4</sub> ), and adrenergic ( $\alpha_2$ ) receptors                                     |
|                   | <b>Psilocybin /Psilocin</b> | 5-HT <sub>1A</sub> , 5-HT <sub>1B</sub> ,<br>5-HT <sub>2D</sub> , 5-HT <sub>2A</sub> , 5-HT <sub>2B</sub> , 5-HT <sub>2C</sub> ,<br>5-HT <sub>3</sub> , 5-HT <sub>5</sub> , 5-HT <sub>6</sub> , 5-HT <sub>7</sub>         | Substrate for 5-HT transporter,<br>agonist of dopaminergic (D <sub>1-5</sub> ),<br>adrenergic ( $\alpha_1$ , $\alpha_2$ , $\beta_1$ ), and H <sub>1</sub> receptors |
|                   | <b>DMT</b>                  | <b>5-HT<sub>1A</sub></b> , 5-HT <sub>1B</sub> , 5-HT <sub>1D</sub> , 5-HT <sub>1E</sub> ,<br>5-HT <sub>2A</sub> , 5-HT <sub>2B</sub> , 5-HT <sub>2C</sub> ,<br>5-HT <sub>5A</sub> , 5-HT <sub>6</sub> , 5-HT <sub>7</sub> | $\sigma_1$ agonist, Substrate for 5-HT transporter                                                                                                                  |
| Ergolines         | <b>LSD</b>                  | 5-HT <sub>1A</sub> , 5-HT <sub>1B</sub> , 5-HT <sub>1E</sub> ,<br>5-HT <sub>2A</sub> , 5-HT <sub>2B</sub> , 5-HT <sub>2C</sub> ,<br>5-HT <sub>5A</sub> , 5-HT <sub>6</sub> , 5-HT <sub>7</sub>                            | Agonist of dopaminergic (D <sub>1-5</sub> ),<br>adrenergic ( $\alpha_2$ , $\beta_1$ , $\beta_2$ ), and H <sub>1</sub> receptors                                     |
| Phenethylamines   | <b>Mescaline</b>            | mostly 5-HT <sub>2</sub> (5-HT <sub>2A</sub> , 5-HT <sub>2B</sub> , 5-HT <sub>2C</sub> )                                                                                                                                  | Some adrenergic antagonism reporter                                                                                                                                 |
| Other/Anaesthetic | <b>Ketamine</b>             | <i>none</i>                                                                                                                                                                                                               | NMDA receptor agonist                                                                                                                                               |

medium-sized interneurons (Jakab & Goldman-Rakic, 1998). The post-synaptic, extra-synaptic localisation of the 5-HT<sub>2A</sub> receptors at a cellular level supports the idea that 5-HT is implicated in paracrine signalling through diffusive routes (Miner et al., 2003). Activation of the 5-HT<sub>2A</sub> receptors leads to a membrane depolarisation associated with a closing of potassium channels leading to an increased frequency of excitatory postsynaptic currents (Aghajanian & Marek, 1997). There is a large body of evidence showing the role of 5-HT<sub>2A</sub> receptors in the regulation of multiple functional responses, as their activation has been linked to changes exploratory behaviour, induces hyperthermia and impulsivity (Halberstadt, 2015; Halberstadt et al., 2020; Nichols & Nichols, 2008; Sharp & Barnes, 2020), as well as the regulation of mood and anxiety disorders as will be discussed in subsection 1.1.3.

The 5-HT<sub>1A</sub> receptor has different properties. First, despite being also linked to the modulation of psychotropic effects (Nichols & Nichols, 2008), 5-HT<sub>1A</sub> receptors activation leads to a hyperpolarisation of the cell and they are thought to be involved in the global regulation of 5-HT signalling *via* the feedback inhibition of 5-HT neurons (Aghajanian, 1995; Riga et al., 2016; Sharp et al., 1997). These

somato-dendritic 5-HT<sub>1A</sub> auto-receptors are located in the dorsal raphe nucleus (DRN) and median raphe nucleus (Riad et al., 2000; Verge et al., 1985). 5-HT<sub>1A</sub> receptors are also post-synaptic hetero-receptors which are expressed in many brain regions, including layers II and VI of the cortex, in the hippocampus and in the entorhinal cortex (Pazos et al., 1985). Studies report that the 5-HT<sub>1A</sub> receptors are implicated in a brain-wide feedback regulation of 5-HT neurotransmission (Sharp et al., 2007). It has been shown that 5-HT<sub>1A</sub> receptors are implicated in the control and regulation of mood, anxiety, sexual behaviour, and cognition likely because of their regulatory role on 5-HT signalling (Sharp & Barnes, 2020).

Different psychedelics bind to the different 5-HT receptors with different affinity. Phenethylamines such as mescaline have been shown to bind more selectively with 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptors, while tryptamines are non-selective 5-HT receptor agonists (Halberstadt, 2015). Even within the tryptamines, there are variations in affinity levels between the 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> receptors. Psilocybin for instance has a higher affinity for 5-HT<sub>2A</sub> receptors while 5-MeO-DMT has a 300-fold higher affinity for 5-HT<sub>1A</sub> receptors compared to 5-HT<sub>2A</sub> (Ermakova et al., 2022; Halberstadt et al., 2012). Ergot-derived psychedelics like LSD also show an affinity for dopaminergic and adrenergic receptors (Halberstadt, 2015). This difference in affinity might account for the differences in psychedelic effects. Indeed, reports suggest that states similar to classical psychedelic-induced ones occur with the anaesthetic and NMDA receptor antagonist ketamine, suggesting that the coactivation of a wide range of receptor might change some aspect of the psychedelic experience but not all of the experience (Miyasaka & Domino, 1968). Furthermore, 5-MeO-DMT and its effects might be considered unique because they are mostly mediated through activation of the 5-HT<sub>1A</sub> receptor, despite discrimination tests in rodents showing no difference compared to other 5-HT<sub>2A</sub> psychedelics (Ermakova et al., 2022).

Studies on psychedelics suggest that the administration of psychedelics to humans and animals elicits strong changes in behaviours. In humans, measures of subjective

changes induced by psychedelics can be assessed through questionnaires like the Mystical Experience Questionnaire (Barrett et al., 2015). These questionnaires assess the capacity of a given drug to elicit a strong and sustained psychedelic experience, which is referred to as the potency of a psychedelic (Halberstadt et al., 2020). In other species, psychedelics elicit a wide range of specie-dependant behavioural responses. Focussing on 5-MeO-DMT, laboratory mice and rats as well as the Syrian hamster (*Mesocricetus auratus*) all show a reduction in locomotor activity, but the Guinea-pig (*Cavia porcellus*) exhibit a dose-dependent increase in locomotor activity (Ermakova et al., 2022). In the goldfish (*Carassius auratus*), surfacing behaviour has been measured which is a classical response to a hallucinogen (Ermakova et al., 2022).

In rodents, the measurement of stereotypical head twitch responses (HTR) has been correlated to the potency of a given psychedelic in humans (Halberstadt et al., 2020). The HTR in rodents is, so far, the only behavioural correlate of the psychedelic state of animals. Further studies on the effects of psychedelics also demonstrate changes in brain activity. Indeed, following an injections or ingestion of psychedelics, electroencephalographic (EEG) recordings show broadband network desynchronisation in both humans and rodents (Murray et al., 2022; Riga et al., 2016, 2018; Vejmola et al., 2021). As the interest in psychedelic research grows, with it grows the need for reliable neurophysiological markers of the psychedelic state going further than subjective reports in humans and behavioural measurements. Electrophysiological recordings might therefore allow a better cross-species comparison.

### 1.1.3 The therapeutic effects of psychedelics

*Psychotomimetics* in the 1950s were marketed and used as tools to explore psychosis, as self-reports from the early twentieth century suggested strong alterations to perception akin to psychotic states (Rucker et al., 2018). The first mention of mescaline being used to enhance the effects of psychotherapy was made in 1936 after the careful observation of 60 participants and the analysis of their subjective reports

(Guttman, 1936). The effects on perception and proprioception of the subjects were noted in great detail. Although instances of strong mystical experiences were reported, the patients still had a sense of reality. These observations suggested differences between the alterations induced by psychedelics compared to the ones of a schizophrenic origin. The state of intoxication observed by Guttman was often followed by “periods in which the subjects ponder over great problems.” This increased period of self-reflection led him to hypothesise a potential increased psychotherapeutic influence (Guttman, 1936). Studies conducted before the UN condemnation explored this route, as detailed in subsection 1.1.2. Most studies suggested a strong therapeutic potential for psychedelics regarding mental health disorders but were mostly underpowered, subject to strong subjective bias, or without control groups (Rucker et al., 2018).

There are also poorly designed recent studies on the therapeutic effect of psychedelics (Rucker et al., 2018). Poorly designed due to their open-label nature, lack of control group, lack of statistical power or subjective results. Nevertheless, most of these studies reproduced what had been observed before the 1970s. Results from open-label trials with ayahuasca suggest psychedelics could help reduce long-term depressive symptoms (Osório et al., 2015; Sanches et al., 2016), and open-label trials with psilocybin suggest that a single dose of psilocyn is sufficient to reduce the symptoms of treatment resistant depression (Carhart-Harris et al., 2016a, 2018a).

Two independent studies conducted on larger samples, following a double-blind, randomised crossover design with convincing controls based on the active placebo niacin or a subthreshold dose of psilocybin successfully showed that a single injection of psilocybin significantly reduced anxiety and depression associated with life-threatening diseases (Griffiths et al., 2016; Ross et al., 2016). All studies also report no adverse effects to the best of their knowledge (Carhart-Harris & Goodwin, 2017; Rucker et al., 2018). Psychedelics are therefore a promising approach, seemingly capable of long-term effects without chronic administration and with low side-effects, together with a rapid-onset improvement in mood in

patients resistant to conventional treatments.

The mechanism underlying the therapeutic effect of psychedelics however is still poorly understood. However, it is speculated that psychedelics induce a state of hyper-plasticity and that therefore the plastic brain would be more susceptible to psychotherapy, as supposed by Guttman in the 1930s. This idea would put psychotherapy as a cornerstone of the therapeutic effect of psychedelics (Brouwer & Carhart-Harris, 2021).

Recent studies on animal models show that indeed classical psychedelics reopen the social reward learning critical period (Nardou et al., 2023). At the neuronal level, psychedelics have been shown to acutely and persistently promote the growth of dendritic spines for up to five days (Shao et al., 2021), consistent with earlier findings that the 5-HT<sub>2A</sub> receptor agonist 4-iodo-2,5-Dimethoxyphenylisopropylamine (DOI) increased brain-derived neurotrophic factor (BDNF) in the hippocampus and cortex (Vaidya et al., 1997). However, these measurements fails to explain the quick onset of positive mood changes reported in human participants (Carhart-Harris et al., 2016a, 2018a, 2018b).

The notion of context might help understand better the therapeutic effect of psychedelics. Before the ban, the notion that non-pharmacological factors such as the set (internal, “mind set”) and setting (external) would influence the psychedelic experience induced by classical psychedelics was theorised first by Al Hubbard, according to Pollan (Pollan, 2019). While it has been established that 5-HT receptors mediate the plasticity effects associated with psychedelics, the content of the psychedelic experience has also been correlated to positive outcomes on mood and anxiety (Carhart-Harris et al., 2018b). This, together with the state of elevated plasticity theorised by Brouwer and Carhart-Harris suggest the existence of a global, highly plastic, brain state, promoted by the activation of 5-HT receptors (Brouwer & Carhart-Harris, 2021).

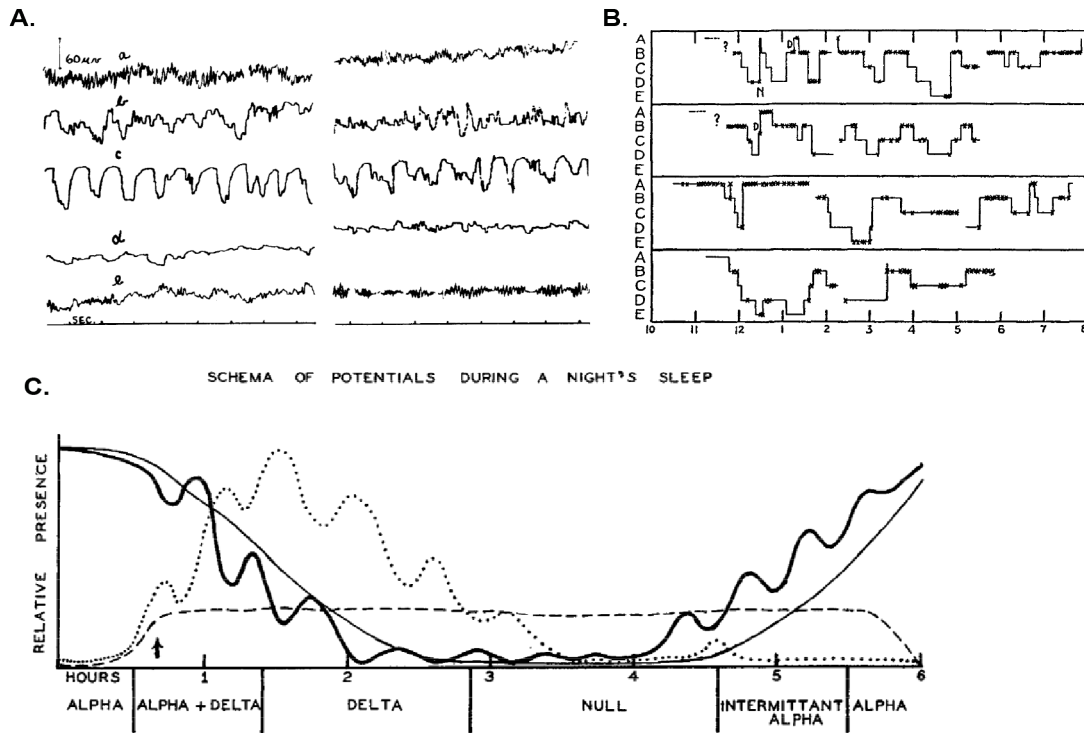
This state, however, has not been well characterised. Changes in brain activity and behaviour allow the discernment of states of vigilance. These will be detailed in the following section. The occurrence of such changes can promote recovery functions through changes in brain plasticity. Thus, it is plausible that psychedelics indeed induce a state of elevated plasticity. It is also plausible that such an altered state shows common behavioural or electrophysiological features akin to other physiological states of vigilance. This would allow its characterisation through well-established definitions and tools.

## 1.2 The study of vigilance states

### 1.2.1 The origin and definition of vigilance states

There are two main vigilance states: wake and sleep. The study of sleep can be traced back to antiquity, with rudimentary evidence of the analysis of dream and sleep self-reports (Askitopoulou, 2015). The characterisation of sleep was close to death. *Thanathos* and *Hypnos*, respectively Gods of Death and Sleep, were twin brothers. This duality however shows a key difference, still used in the present time: sleep is a reversible state (Cirelli & Tononi, 2008).

Physiologists of the nineteenth century reframed sleep as a brain-centred state. Wundt proposed in 1890 that different brain processes occurred during wake and sleep and Alfred Maury suggested that the function of sleep was linked to memory processes (Hobson, 1988). However, like the early research on psychedelic drugs, sleep was typically analysed with self-reports and self-experimentation, leading to strong bias and erroneous conclusions. The subjective analyses were poorly reproducible. In the early twentieth century however, theories became more diverse. The reports gradually changed to a more medical vernacular using a more scientific approach and reproducible questionnaires. Sleep was then argued to be a state occurring across vertebrate species, as well as invertebrate and even plant species, as a resting state, relying on strictly behavioural markers, such as a stereotypical



**Figure 1.4: The early ages of sleep research.** **A.** From Blake et al. (1938): “Potential patterns through the night on three subjects. a. “Alpha” rhythm of wakefulness. b. “Alpha+delta” period of light sleep. c. “Delta” period of deep sleep. d. “Null” period of light sleep. e. “Alpha” rhythm of wakefulness. **B.** From Loomis et al. (1937): “Night sleep records of four individuals, the first two non-alpha types [i.e.: individuals not showing alpha rhythm], the last two alpha type showing changes in states of sleep, A, B, C, D, E (vertical) during the hours of the night (horizontal). The crosses represent major movement. N, noise, D, a dream.” Note that the occurrence of dream is linked with the B phase of light sleep, as the first stages of light sleep and paradoxical sleep were thought to be the same phase. **C.** From Blake et al. (1938): “Predominance of brain potentials through the night. Alpha waves (black heavy line) in per cent presence; 14 per sec. waves (dashes) in per cent presence and delta waves (dots) in extent of predominance. Oral temperature (black thin line). Below the stages of sleep are indicated. Record begun at time of retiring arrow indicates beginning of sleep. Wavy lines represent changes in waves due to shift of state of sleep. Depth of sleep by auditory response method roughly parallels delta curve.” This figures shows that sleep was thought to be a continuous state through which stages follow one another linearly.

resting position (Piéron, 1913).

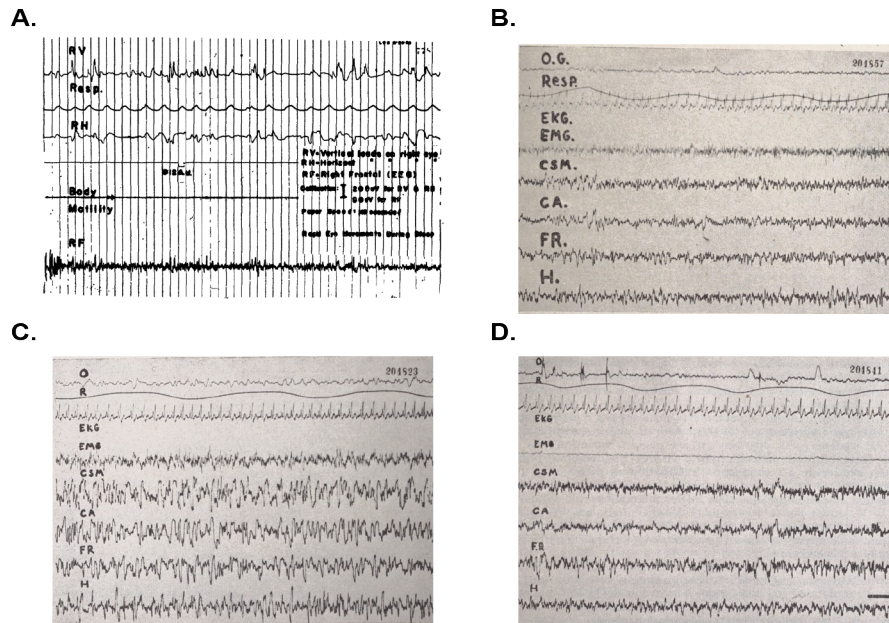
Relying on the notion that cerebral architecture allows the measurement of local and global variations of the electrical field potentials (Buzsáki et al., 2012), the development of the electroencephalogram by Berger in 1929 allowed the non-invasive



measure of brain potentials. This expanded the field of electrophysiology, allowing measurement in healthy humans as well as comparative measurements in animals. The 1930s brought the notion that alteration of vigilance through the reduction of sensory inputs can have a prominent effect on brain rhythms (Adrian & Matthews, 1934; Adrian & Yamagiwa, 1935), and further demonstrated that it was possible to determine different brain states and sleep stages based on features observed in EEG recordings (Figure 1.4). Early EEG work identified four sleep stages. First, a stage with 10 Hz trains labelled alpha waves. Second, a stage of low voltage and rolling of the eyes often associated with dreams called the “null” phase. Third, a stage composed of irregular spindle-shaped bursts of activity around 14 Hz, labelled “sleep spindles”. Finally, a stage showing delta waves, which are large waves of between 0.5 Hz and 3 Hz with a high amplitude (H. Davis et al., 1937; Hess et al., 1953; Loomis et al., 1935; Loomis et al., 1937; Rheinberger & Jasper, 1937). These studies measured sleep depth and correlated it with the occurrence of the delta wave. Sleep depth was assessed by measuring the arousal threshold, represented by the minimal stimulus intensity needed to wake the participants up (Blake & Gerard, 1937; Blake et al., 1938).

Further studies on the “null” phase revealed a more complex physiology. Its rare occurrence during afternoon naps suggests this phase depends on other sleep stages. Its prominence in babies also pointed to a potentially crucial developmental function (Blake & Gerard, 1937; Blake et al., 1938; Loomis et al., 1935, 1936). Further work by Aserinsky and Kleitman in 1953 described the recurrence of the “null” phase in humans, on average every 1 hour and 40 minutes of sleep, together with the occurrence of rapid eye movements (REM) (Figure 1.5.A).

Its occurrence in animals was first demonstrated by Rimbaud and Dement (Aserinsky & Kleitman, 1953; Dement, 1958; Rimbaud et al., 1955). Later on, the work of neurophysiologist and anatomist Michel Jouvet further defined this sleep stage, allowing sleep to be fully subdivided into 2 substates: one where slow waves of high amplitude were predominant and labelled slow wave sleep (SWS), and one



**Figure 1.5: The discovery of a paradoxical state.** **A.** From Aserinsky and Kleitman (1953): “Sample record exhibiting rapid eye movements in a sleeping subject. RV = vertical leads on right eye ; RH = horizontal leads on right eye ; RF = right frontal (EEG). Calibration: 200  $\mu$ V for RV & RH; 50  $\mu$ V for RF; paper speed : 10 sec.” **B-D.** From M. Jouvet et al. (1959c) (translated): **B.** “Recordings of an awake, chronically implanted cat. O.G.: ocular movement acquired with electrodes located by the left eyelid. Resp: Breathing rate, EKG: electrocardiogram, EMG: electromyograph from the nuchal muscles, CSM: Sensorymotor cortex, CA: Auditory cortex, FR: reticular mesencephalic formation, H: ventral hippocampus. Calibration: 1 second 50  $\mu$ V.” **C.** “Recordings of a chronically implanted cat in deep sleep”. **D.** “Recording of a chronically implanted cat in the “paradoxical phase.” Note the rapid eye movement, the breathing rate acceleration, the slowing down of the electrocardiogram and the complete absence of muscular activity.”

where the EEG showed wake-like fast activity despite a high arousal threshold labelled paradoxical sleep (PS) (M. Jouvet & Michel, 1959; M. Jouvet et al., 1959c) (Figure 1.5.B-D).

### 1.2.2 The current understanding of vigilance states

The current definitions for wake and sleep account for the behavioural changes typical of each state as well as physiological features (Cirelli & Tononi, 2008). From a behavioural point of view, wake is a state of vigilance characterised by high muscle activity and the expression of various behaviours, allowing the organism to react

to stimuli. Sleep, on the other hand, is a reversible state of quiescence with low responsiveness to external stimuli, where the animal expresses a species-specific body posture usually in a specific location (Campbell & Tobler, 1984; Piéron, 1913). Whether sleep or wake is a default state can be debated. The reversibility of sleep suggests that wake is a default state, but the same can be said about sleep (Meddis, 1977).

Further studies on the vigilance states showed that sleep can be divided into two main substates in a vast majority of species (Blumberg et al., 2020). While it has been shown that behavioural features are variable throughout the animal kingdom, PS is still distinguishable from SWS through muscle inactivity, REMs, twitches of the tip of the limbs, and a higher arousal threshold assessed through the presentation of waking stimuli (M. Jouvet et al., 1959c).

Electrophysiological measurements, however, allow for a clearer distinction between vigilance states. The wake state can be characterised by a fast EEG activity of low amplitude, with a higher expression of higher EEG frequencies ( $> 30$  Hz). Measuring muscle activity with electromyographic (EMG) recordings reveals that the subject is highly active. During SWS, waves of large amplitude in the delta frequency range (0.5-4 Hz) are strongly expressed, together with transient spindles (10-15 Hz) while the muscle tone on the EMG is greatly reduced (Adamantidis et al., 2019; Steriade, 2000). PS was named due to the paradoxically wake-like cortical activity with a high activity in the theta frequency range (7-10 Hz) in the stage of sleep with the highest arousal threshold, while the EMG showed a complete muscle atonia (Dement, 1958; M. Jouvet et al., 1959c).

As suggested in the early twentieth century and supported by more recent observations, it appears that most, if not all, animals express sleep (Blumberg et al., 2020; Cirelli & Tononi, 2008; Piéron, 1913). Sleep-like states have been observed in fish (Tobler & Borbély, 1985; Zhdanova et al., 2001), cockroaches (Tobler & Neuner-Jehle, 1992), honeybees (Kaiser & Steiner-Kaiser, 1983; Sauer et al., 2003),

and sleep substates have been observed in bearded dragons (Norimoto et al., 2020) and several species of birds (Klein et al., 1964; Ungurean & Rattenborg, 2024; Ungurean et al., 2021), as well as in arthropods (Roßler et al., 2022). However, comparative works suggest that there might be as many different sleeps as there are species (Blumberg et al., 2020; Campbell & Tobler, 1984; Cirelli & Tononi, 2008). Owls for instance produce electrophysiological markers of PS but fail to produce rapid eye movements or muscle atonia (Blumberg et al., 2020).

Forms of sleep are also present in marine mammals and migratory birds despite the environmental pressure against the development of complete states of rest (Blumberg et al., 2020; Cirelli & Tononi, 2008). The observation of unihemispheric sleep, measurement of sleep-like slow waves in one brain hemisphere while the other one is undoubtedly awake, would theoretically allow the animal to rest while still being able to navigate its environment (Rattenborg, 2017), a crucial element for marine mammals. Indeed, such animals can only stay in apnea for a fixed amount of time and exhibits surfacing behaviour and it is thought that sleep would greatly hinder this type of behaviour. The aquatic environment is also thought to be responsible for the suppression of PS, as said suppression of PS has been observed in cetaceans as well as fur seals when sleeping underwater. Such suppression might be due to muscle atonia and the suspension of thermoregulation, hallmark features of PS, thought to be harmful in submerged conditions (Blumberg et al., 2020; Lyamin et al., 2018; Madan & Jha, 2012). However, with SWS being present unihemispherically in many cetaceans, it is not unlikely that PS could be expressed unihemispherically and not classified as such due to behavioural observations and the typical wake-like EEG signals (Rattenborg et al., 2000).

Humans are diurnal with a stereotypical sleep/wake cycle throughout the 24 hour period, with sleep mostly occurring in one block during the night (Blake et al., 1938; Loomis et al., 1937)<sup>2</sup>. On average, human sleep cycles between SWS and PS

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<sup>2</sup>However, it is worth noting that the consolidated monophasic nature of human sleep is being debated by anthropologists according to recent reports. Indeed, pre-industrial era journals suggest the occurrence of two periods of sleep during the night (Ekirch, 2016), but the study of

every one hour and 40 minutes (Aserinsky & Kleitman, 1953). SWS can be further subdivided into three substates mostly based on electrophysiologic criteria : (i) N1, a stage of mixed frequency oscillations with occasional slow rolling of the eyes and alpha waves in the EEG; (ii) N2, a deeper stage with the occurrence of sleep spindles as well as other transient electrophysiological events like K-complexes; (iii) N3, the deepest stage of SWS mostly composed of large amplitude delta waves in the EEG, with an estimated SWS duration of two to three hours per SWS episode (“Rules, Terminology and Technical Specifications”, 2017). The occurrence of delta waves, or slow waves, in humans has been correlated with subjective sleep depth (A. M. Stephan et al., 2021). PS, on the other hand, has been associated with dreams early on (Aserinsky & Kleitman, 1953; Blake et al., 1938), but recent studies shows that conscious, dream-like experiences are not confined to the PS and might be anti-correlated to the slow wave activity power (Siclari et al., 2017).

Cats have been historically used as the animal model to study sleep (Dement, 1958; M. Jouvet et al., 1959c). However, rodents and more specifically mice as the ones used in this thesis, have also proven themselves to be a reliable model. Laboratory mice are nocturnal, polyphasic sleepers (Campbell & Tobler, 1984; Valatx & Bugat, 1974). Their sleep is heavily fragmented, with an estimated SWS episode duration of typically less than 10 minutes and an average of 30 SWS to PS transitions in 24 hours (Campbell & Tobler, 1984; Franken et al., 1998; Valatx & Bugat, 1974). SWS in rodents is not subdivided into several substages, with the occurrence of large EEG slow waves marking the beginning of SWS.

### 1.2.3 Slow waves as a hallmark feature of sleep

The occurrence of global slow waves on EEG recordings is arguably the most notable electrophysiological feature of sleep. The measurement of the fluctuations of electrical potentials on the EEG are widespread and reflect the synchronicity of a wide network surrounding the electrode (Buzsáki et al., 2012). It is also possible pre-industrial societies failed to show any evidence of this bi-phasic sleep (Yetish et al., 2015).

to insert intracortical electrodes and, with electrodes of the right configuration and impedance, record fluctuations of the local field potential (LFP) and neuronal activity (multiunit activity, MUA) provided the correct filter is put in place (Buzsáki et al., 2012; Destexhe et al., 1999).

Using intracortical and subcortical recordings, it has been shown that EEG slow waves correspond to a bistable state of the network (Steriade et al., 1993a; Wilson & Groves, 1981). During SWS in rodents, this bistability is reflected in Up- and Down- states of neuronal firing patterns (Vyazovskiy et al., 2009b). The Up-state, or ON-period, corresponds to a moment of depolarised neuronal membrane potential, resulting in a high spiking probability. The Down state, or OFF-period, corresponds to moments of hyperpolarised membrane potentials thus resulting in a period of neuronal quiescence with little to no neuronal firing. The rhythmic, oscillatory pattern between ON- and OFF- periods corresponds to the peaks of slow waves observed on LFP recordings (Nir et al., 2011; Poulet & Petersen, 2008; Vyazovskiy & Harris, 2013). Such oscillatory patterns are not confined to the neocortex, as recordings in subcortical regions also reported periods of synchronised neuronal silences in the thalamus (Steriade et al., 1993a) as well as the cerebellum, hippocampus and amygdala (Collins et al., 2001; Roš et al., 2009; Wolansky et al., 2006).

The mechanisms responsible for the generation and maintenance of slow waves is still debated. Early studies on *encéphale isolé*<sup>3</sup> cats suggested that slow waves were the default state of the telencephalon and therefore should have a neocortical origin (M. Jouvet & Michel, 1958, 1959). Thalamic lesions, as well as *in vivo* isolated cortical slabs and *ex vivo cortical* preparations showed the appearance of slow waves, supporting these earlier claims (Sanchez-Vives & McCormick, 2000; Steriade & McCarley, 2013; Timofeev et al., 2000). Recent observations also suggest that

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<sup>3</sup>French for *isolated encephalon*, technique where neuronal connections were severed between the telencephalon and hindbrain, leaving the forebrain disconnected from stimulus rising outside of itself. This technique was performed *in vivo* and had an elevated mortality rate (Bremer, 1935; Moruzzi & Magoun, 1949).

cortical layer V is involved in the cortical generation of slow waves (Krone et al., 2021; Sanchez-Vives & McCormick, 2000). The thalamus however should not be removed from the equation altogether. It has been shown that cortical Up- and Down- states correlated with thalamic Up- and Down- states (David et al., 2013), and the inhibition of the thalamus during anaesthesia and natural sleep reduced the slow wave peaks (David et al., 2013). Should slow waves have a complete cortical origin, their modulation and maintenance are strongly mediated by the cortico-thalamic network.

As mentioned previously, the amplitude of slow waves in humans can be correlated with subjective sleep depth (A. M. Stephan et al., 2021). Furthermore, slow wave activity is highly correlated with sleep pressure, as the incidence of high amplitude slow waves depends on preceding sleep/wake history (Borbély, 1982; Borbély et al., 2016; Vyazovskiy et al., 2009b, 2011a). These observations suggest that slow waves are not only a hallmark feature of sleep, but also might be the mechanism underlying a hypothesised restorative function of sleep (discussed later in subsection 1.3.3).

Slow waves have been described mostly in sleep and anaesthetic states as a global phenomenon (Timofeev et al., 2000; Vyazovskiy et al., 2009a, 2009b, 2011a). However, slow waves are not an electrophysiological feature constrained to the SWS state. Indeed, early reports using EEG showed that local slow waves were occurring around lesion sites, suggesting some potential restorative or protective functions (Walter, 1936). Further evidence also shows local intrusion of sleep-like slow waves during wakefulness in humans and rodents (Andrillon et al., 2021; Borbély et al., 1984; Nir et al., 2011; Vyazovskiy et al., 2011b). In stroke patients, local slow waves have been reported near the lesion site confirming the observation made by Walter (1936), thus supporting the notion that slow waves have some restorative properties (Sarasso et al., 2020). The occurrence of slow waves during wakefulness has been linked to a diminution of attention and behavioural performance in rodents and humans (Andrillon et al., 2021; Vyazovskiy et al., 2011b). Taken together,

these observations suggest that the function of slow waves, should there be any, goes beyond the scope of sleep.

Sleep deprivation, while increasing slow wave activity during sleep, also increases the occurrence of bursts of local slow waves during episodes of wake (Borbély et al., 1984; Vyazovskiy et al., 2011b). Experiments involving the administration of atropine, a cholinergic antagonist, report sleep-like slow waves during wakefulness which did not alter the sleep-deprivation-induced increase in slow wave activity (Qiu et al., 2015). This suggests that while sleep processes may rely on slow wave activity, slow waves are a phenomenon not bound to sleep. Local slow waves, as argued in Krone and Vyazovskiy (2020), might therefore only be a marker of disconnection, but it does not mean that they do not coincide with any restorative processes. It is entirely plausible that, when disconnected, the cortical networks might revert to a default, disconnected state rich in slow waves during which restorative or cellular maintenance processes take place, as argued by Vyazovskiy and Harris (2013), thus promoting a more efficient network when the disconnected state is lifted.

## 1.3 Regulation and functions of sleep

### 1.3.1 The circuitry of vigilance states

#### 1.3.1.1 The general approach to wake-promoting areas

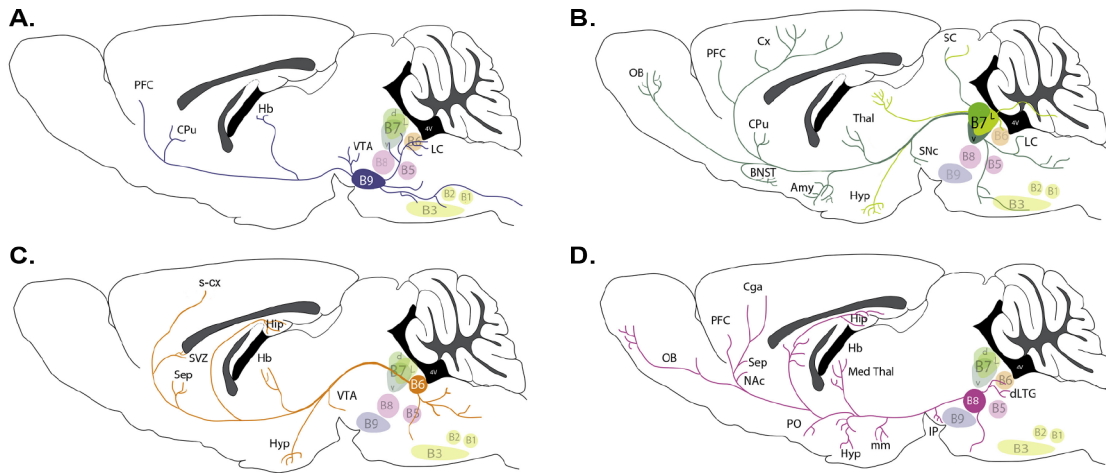
In 1917, Von Economo described cases of *Encephalitis lethargica* and the associated lethargic symptoms (von Economo, 1929). The increased sense of sleepiness was assigned to brain lesions in the substantia nigra and the anterior hypothalamus which allowed Von Economo to correctly identify these structures as wake promoting areas (von Economo, 1929). Further studies on post influenza encephalitis-induced insomnia suggested the existence of a hypnogenic centre in the hypothalamic preoptic area (von Economo, 1930). These results implied that the regulation of vigilance states was in the brain. His work, coupled with the birth of the EEG, allowed for a better understanding of the regulation of sleep and wake states.



Bremer's work on the *encéphale isolé* later showed that the slow waves could be a default activity mode of the brain and suggested the existence of a circuit responsible of wake-like cortical activity (Bremer, 1936; M. Jouvet & Michel, 1958; Magoun, 1950). It is by looking for a wake-promoting centre in the brainstem that Jouvet characterised PS (M. Jouvet et al., 1959b, 1959c). More recent work showed that wake, SWS, and PS are regulated by an extensive neuronal circuitry relying on the activation and inactivation of numerous subcortical nuclei, mediated by the mobilisation of several distinct neuromodulator systems and their associated neurotransmitters (Fort et al., 2009).

The monitoring of state-specific activity of cellular populations allowed for a better understanding of state regulation and circuitry by identifying cerebral structures responsible for the onset or maintenance of vigilance states (Fort et al., 2009). The neurotransmitters systems involved with arousal rely on the monoaminergic circuits of noradrenaline (NA) from the locus coeruleus (LC), serotonin (5-HT) from the dorsal raphe nucleus (DRN), acetylcholine (Ach) from the pontine tegmentum, but also other circuits including the hypocretin-containing neurons of the lateral hypothalamus and the dopaminergic system (Eban-Rothschild et al., 2016; Fort et al., 2009; Sakai, 2011).

Cortical and hippocampal levels of Ach are higher during wake and PS than during SWS, and NA containing neurons fire at a higher rate during waking, and then lower their firing rate with sleep depth (Aston-Jones & Bloom, 1981; Carlson, 2016; Marrosu et al., 1995). Firing rates of NA neurons in the LC have also been correlated with an infra-slow oscillatory rhythm of arousal levels (Carro-Domínguez et al., 2023; Osorio-Forero et al., 2021). Fiber-photometric studies showed that the dopaminergic system was involved in the onset and maintenance of arousal, as dopaminergic neurones of the VTA fire in a state-specific manner, promoting wakefulness, and their inhibition led to sleep-specific behaviours (Eban-Rothschild et al., 2016). 5-HT cell firing has also been shown to decrease with the occurrence of sleep and quiescent behaviours (Trulson & Jacobs, 1979). Therefore,



**Figure 1.6: Projections of the 5-HT system**, adapted from Muzerelle et al. (2016) and Soiza-Reilly and Gaspar (2020). **A.** Projections from the supramedian and pedunculopontine nuclei (B9) **B.** Projections from the lateral (B7L, light green) and the ventral (B7v, dark green) subdivisions of the dorsal raphe. **C.** Projections from the caudal (B6) subdivisions of the dorsal raphe. **D.** Projections from the median raphe nuclei (B8) and pontine raphe nuclei (B5).

the monoaminergic nuclei, with their cortical-wide projections, are part of what has been labelled the ascending arousing pathways, and are thought to mediate the high levels of activity observed in cortical and thalamic networks during wakefulness and the maintenance of elevated levels of arousal (Fort et al., 2009).

### 1.3.1.2 The serotonergic system as a wake-promoting system

As a neuromodulatory system, the 5-HT system expresses a total of fourteen 5-HT receptor subtypes (Table 1.2) all implicated in the regulation of complex behaviour (Nichols & Nichols, 2008; Sharp & Barnes, 2020). The diversity of the 5-HT system is also found at the level of the 5-HT containing neurones, whose localisation allows a subdivision into clusters with projections covering all of the cerebral structures (Muzerelle et al., 2016; Parent & Descarries, 2020; Soiza-Reilly & Gaspar, 2020) (Figure 1.6). 5-HT receptors are widely distributed across the brain in both cortical and subcortical regions, with a subtype specificity at the resolution of cortical layers, as exemplified in subsection 1.1.2 with the 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> receptors (Sharp & Barnes, 2020). 5-HT is a diffusive neurotransmitter, such that many 5-HT

containing neurones do not directly project to a synapse but rather release 5-HT from presynaptic varicosities, suggesting 5-HT is important for the modulation of local networks as well as individual cells (Bunin & Wightman, 1998).

Although 5-HT neurons are part of the reticular ascending arousing pathway and their firing correlates with wake states (Trulson & Jacobs, 1979), the relationship between 5-HT and sleep is unclear. It has been suggested that the 5-HT system, as a whole, might be important for some hypnogenic mechanisms (M. Jouvet, 1999). In support of this idea, an inhibition of the DRN through the 5-HT<sub>1A</sub> autoreceptors has been shown to increase PS (Portas et al., 1996). Experiments involving either the activation or the inactivation of 5-HT receptors have both been shown to alter the sleep/wake cycle in a quantitative manner by increasing the amount of time spent in wake and promoting the occurrence of short periods of arousal during sleep (Monti, 2011; Thomas et al., 2022). However, the notion that potentiating the 5-HT system could result in a state of vigilance not corresponding to the current definition of wake or sleep has not yet been considered.

### **1.3.1.3 The sleep switch and regulation of sleep states**

The circuitry underlying sleep states was first explored in the 1930s, when the ventrolateral preoptic area of the hypothalamus was first identified as a hypnogenic centre (Moruzzi & Magoun, 1949; Nauta, 1946; von Economo, 1930). Originally, the co-existence of both a hypnogenic centre and an arousal promoting pathway was debated as it was shown that slow waves could occur in an isolated cortex devoid of a hypnogenic centre (Bremer, 1936; M. Jouvet & Michel, 1958, 1959; Magoun, 1950). However, electrophysiological and neuroanatomical studies showed that neuronal activity increased with SWS onset in the the ventrolateral preoptic area (VLPO), thus bringing evidence that the VLPO was a potential hypnogenic centre (Gong et al., 2004).

VLPO neurons are GABAergic neurons projecting towards wake-promoting regions such as hypocretin containing neurons of the lateral hypothalamus, as well

**Table 1.2: 5-HT receptor subtypes and expression.** Adapted from Sharp and Barnes (2020) and Nichols and Nichols (2008). To note: "At the macro-level, 5-HT receptors are widely distributed, with specific brain regions having distinct complements of 5-HT receptor subtypes. [...] It is clear that expression of specific 5-HT receptors is not restricted to a particular neuron type. For example, within the cerebral cortex, hippocampus and amygdala, 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors are expressed both on pyramidal neurons (glutamatergic) as well as major classes of inhibitory (GABAergic) interneurons (in particular those expressing parvalbumin). [...] Collectively, evidence suggests that the pattern of 5-HT receptor distribution is largely similar across vertebrate species." Further studies on human and monkey reveals similar placement, with 5HT<sub>2A</sub> receptor being expressed in thalamic nuclei and the upper layer V and layer II of the anterogenuel, enthorinal and frontal cortices.

| Type                                  | Subtype | Expression                                                                                                                                | Comments                                          |
|---------------------------------------|---------|-------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|
| <b>5-HT1</b><br>(G <sub>auo</sub> )   | 5-HT1A  | 5-HT neurons (soma and dendrites), neocortex, limbic areas                                                                                |                                                   |
|                                       | 5-HT1B  | 5-HT neurons (soma and terminals), basal ganglia, mesolimbic pathways, trigeminal nucleus                                                 |                                                   |
|                                       | 5-HT1D  | Midbrain raphe 5-HT neurons, trigeminal nucleus                                                                                           |                                                   |
|                                       | 5-HT1E  | <i>Not expressed in rodents</i>                                                                                                           | Limited expression outside the CNS, gene products |
|                                       | 5-HT1F  | Midbrain raphe 5-HT neurons, trigeminal nucleus                                                                                           |                                                   |
| <b>5-HT2</b><br>(G <sub>aq/11</sub> ) | 5-HT2A  | Neocortex, limbic areas, basal ganglia, mesolimbic pathways                                                                               |                                                   |
|                                       | 5-HT2B  | Midbrain raphe 5-HT neurons                                                                                                               | Mostly expressed in the liver, kidney, stomach    |
|                                       | 5-HT2C  | Midbrain raphe 5-HT neurons, neocortex, limbic areas, basal ganglia, mesolimbic pathways, paraventricular and arcuate hypothalamic nuclei | Limited expression outside the CNS                |
| <b>5-HT3</b><br>(Ion channel)         |         | Neocortex, limbic areas, dorsal vagal complex                                                                                             |                                                   |
| <b>5-HT4</b><br>(G <sub>as</sub> )    |         | Neocortex, limbic areas, mesolimbic pathways                                                                                              |                                                   |
| <b>5-HT5</b><br>(G <sub>auo</sub> )   | 5-HT5A  | Midbrain raphe 5-HT neurons, raphe GABA interneurons                                                                                      |                                                   |
|                                       | 5-HT5B  |                                                                                                                                           | Gene products                                     |
| <b>5-HT6</b><br>(G <sub>as</sub> )    |         | Neocortex, limbic areas                                                                                                                   | Limited expression outside the CNS                |
| <b>5-HT7</b><br>(G <sub>as</sub> )    |         | Suprachiasmatic nucleus                                                                                                                   |                                                   |

as the DRN, and LC (Fort et al., 2009). Such observations support the notion of a “sleep switch” structure that, when activated, would inhibit wake promoting areas. The activation of the sleep switch could be regulated by processes which will be described in subsection 1.3.2. The idea however that sleep operates solely with a switch can be disputed. Indeed, while there seems to be a clear distinction between sleep and wake states, sleep shows nuanced behaviour and can be divided into substates which strongly implies the existence of more complex regulatory mechanisms.

The circuitry underlying PS as a substate has been extensively studied by the laboratory of Michel Jouvét in the 1960s with lesion work. As opposed to the observation of physiological sleep in humans or animals (Aserinsky & Kleitman, 1953; Dement, 1958), Jouvét’s work on animals was both anatomical and electrophysiological, showing the existence of a rhombencephalic (or hindbrain) hypnogenic centre (M. Jouvét & Michel, 1959; M. Jouvét et al., 1959b, 1959c).

His works put SWS and PS on opposite grounds. First, based on electrophysiological recordings: SWS shows slow cortical EEG activity while PS shows wake-like, fast EEG activity. Second, in terms of generative centres: SWS with a putative telencephalic origin as opposed to the rhombencephalic origin of PS. In the pons, experiments conducted on the sublaterodorsal nucleus (SLD) showed that neurons would only fire while in PS, thus labelling them PS-on neurons (Clément et al., 2011; Sapin et al., 2009). Evidence suggests that the state-specific activity of these neurons is maintained through reciprocal inhibition between the SLD PS-on neurons and PS-off neurons located in the periaqueductal grey. The PS-off neurons have been shown to maintain a tonic GABAergic inhibition of SLD neurons thus preventing the occurrence of PS (Fort et al., 2009).

It is thought that at PS onset, GABAergic neurons from the posterior hypothalamus inhibit wake-promoting neurons as well as the PS-off neurons from the periaqueductal grey, thus lifting the inhibition on GABAergic and glutamatergic

PS-on neurons which, in turn, promotes PS and inhibits wake-promoting nuclei like the DRN and the LC (Fort et al., 2009).

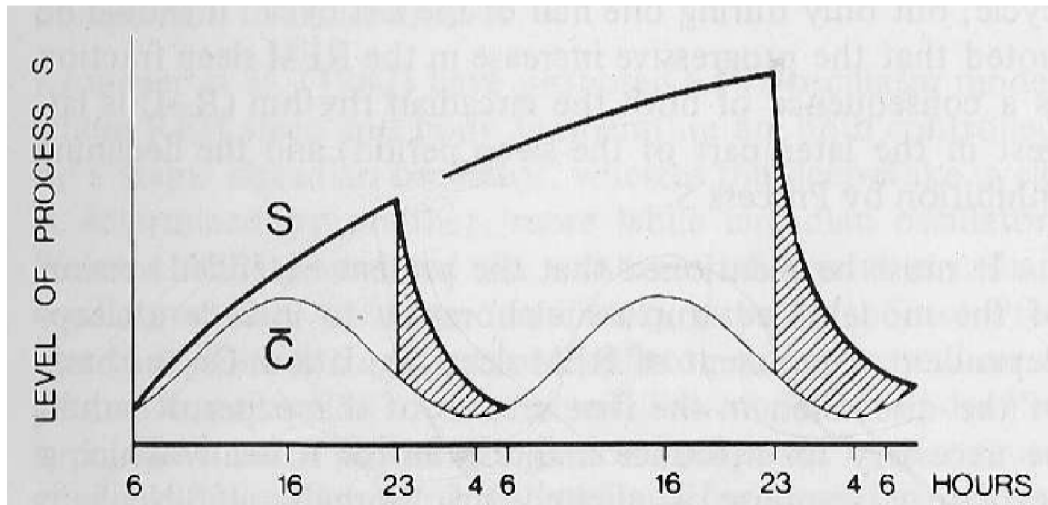
The elevated theta rhythm during PS is likely due to GABAergic neurons located in the medial septal nucleus projecting to the hippocampus (Boyce et al., 2016), and evidence suggests that the overall cortical activation is mediated through an activation of the claustrum (Renouard et al., 2015). It has been argued that, because the activated structures are part of the limbic system, heavily implicated in the regulation of emotional memory, PS might have a role in the regulation and processing of emotions (Luppi et al., 2022).

### 1.3.2 The regulation of sleep

#### 1.3.2.1 The subprocesses of sleep regulation

The control of sleep and wake states therefore relies on extensive and intertwined neuronal networks. Throughout this introduction, sleep has been established as a reversible state with defined behavioural and electrophysiological features. Sleep is also homeostatically regulated (Campbell & Tobler, 1984; Cirelli & Tononi, 2008), represented by the concept of sleep pressure. In previous paragraphs, it was demonstrated that sleep/wake history, mostly illustrated by extended periods of sleep deprivation, can increase the power of EEG delta waves as well as promote the intrusion of sleep-like slow waves during wake states (Vyazovskiy et al., 2011a).

However, the existence of diurnal and nocturnal animals implies the existence of a separate process (or processes) determining the optimal timing for the occurrence of sleep. Over a 24-hour period, most environments at the surface of the planet undergo major shifts in conditions due to the rising and setting of the sun. The ecological hypothesis of sleep suggests that anticipating major shifts in the environment, and behavioural adaptations to these shifts, could be advantageous and allows the development of highly specific behaviours. An adaptation from this anticipation exists in the form of pacemakers entrained by external cues (*Zeitgebers* (ZT),



**Figure 1.7: The two-process model of sleep regulation.** From Borbély (1982). Illustration of the 2-process model of sleep regulation. Process C oscillated with the circadian rhythm at 1 cycle per 24 hours. Process S increases with time spent awake and decreases exponentially with time spent asleep.

timekeepers). Such pacemakers persist without these external cues, thus promoting the maintenance of circadian<sup>4</sup> rhythms.

The interplay of a circadian rhythm and a homeostatic need for sleep has been developed as the two-process model of sleep regulation (Borbély, 1982; Borbély et al., 2016). This model suggests the interaction between a circadian process, process C, and a homeostatic process, process S. Process C is the more constant and rhythmic, dictated by the circadian master clock of the organism. Process S infers the existence of a need for sleep which would increase with the time spent awake (Figure 1.7) (Borbély, 1982).

### 1.3.2.2 Process C and the circadian oscillator

Experiments reported that lesions of the suprachiasmatic nucleus (SCN) induced arrhythmicity in circadian-led behaviour such as drinking and locomotor activity (Moore & Eichler, 1972; F. K. Stephan & Zucker, 1972). Further studies including the transplant of wild-type SCN cells into mutant arrhythmic hamsters showed that

<sup>4</sup>from the Latin *circa* around and *dies* day

a restoration of circadian rhythm (Ralph et al., 1990) led to the identification of the SCN as the master clock of the circadian bodily rhythms, under the influence of visual stimulation from the retinohypothalamic tract (Moore & Lenn, 1972). It has been shown that clock cells in the SCN can all be independent circadian pacemakers. SCN neurones exhibit a circadian rhythmicity in their firing patterns unsynchronously from one another, despite apparent functional connections (Welsh et al., 1995). Therefore, this implies that clock-related processes are intracellular, but that the influence of zeitgebers is necessary to keep the clock synchronised and entrained to the environment.

The discoveries of the mechanisms underlying the circadian rhythm and the processes of the inner clock were awarded the 2017 Nobel Prize (Jeffery C. Hall, Michael Rosbash, Michael W. Young). The intracellular circadian pacemaker relies on the expression of clock genes and transcription factor *Period1-2* and *Cryptochrome1-2*, and *Bmal1* and *Clock* whose reciprocal inhibitory/excitatory interactions represent the backbone of mammalian circadian pattern generations (Reppert & Weaver, 2002).

It has been shown that SCN neurons activity is sensitive to external light stimulation (Meijer et al., 1998). Furthermore, experiments showed that the SCN projects directly to the VLPO (Novak & Nunez, 2000). C-Fos labelling experiment also showed that the rhythmic activation of the VLPO throughout the day was similar to that observed in the SCN, suggesting a synchronous activity between the SCN and the VLPO (Novak & Nunez, 1998). Therefore, it is possible to conclude that sleep is under strong circadian gating from the SCN, itself synchronised mainly by signals originating from photic zeitgebers and the retina through the retinohypothalamic tract.



### 1.3.2.3 Process S and the need for sleep

The notion of homeostasis was theorised by the physiologist Claude Bernard in the middle of nineteenth century following observations of a drive towards constant and stable conditions in the internal environment of animals. These conclusions were made during vivisections, by observing that lesions of the liver or injections of the 4th ventricle led unstable glycemia (Gross, 1998). The word “homeostasis<sup>5</sup>” was coined much later in the 1920s by the Physiologist Walter Cannon. The homeostatic regulation of sleep therefore infers that the longer time is spent awake, the greater the need for sleep will be. Such an idea is supported by the observation of sleep rebounds, longer episodes of sleep showing less interruption and arousal, occurring after sleep deprivation (Borbély & Neuhaus, 1979). A direct consequence of sleep deprivation is the increase of slow wave activity on the EEG, followed by a gradual decrease during subsequent sleep (Borbély & Neuhaus, 1979; Tobler & Borbély, 1986; Vyazovskiy & Tobler, 2012), thus making slow wave activity an excellent measure for sleep pressure as part of homeostasis.

Evidence suggests that adenosine is heavily involved in the pharmacological mechanisms underlying the homeostatic regulation of sleep (Benington & Heller, 1995; Fort et al., 2009). Studies with caffeine show that blocking the adenosine receptors A1 and A2 acts as a stimulant and reduces sleepiness (Snyder, 1985). The continuous infusion of an A2 receptor agonist during 6-hour also dramatically increased the proportion of SWS and PS in rats (Sato et al., 1996) and local injection of an A2 receptor agonist near the hypnogenic VLPO increased neuronal activity in the VLPO (Scammell et al., 2001).

The rise in concentration of adenosine has been linked to metabolic activity in cells, and it is therefore hypothesised that sleep, relying on the fluctuation of adenoside levels, can help in maintaining the brain’s global energy homeostasis by promoting low levels of neuronal activity (Porkka-Heiskanen & Kalinchuk, 2011).

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<sup>5</sup>from Greek *homoios*, similar, and *stasis*, standing still

The two-process model of sleep regulation brings the circadian and homeostatic regulation of sleep together (Borbély, 1982, 2022). Indeed, as both the circadian rhythm mediated by the SCN and putative pharmacological markers of sleep homeostasis both result in the activation of sleep promoting neurones in the VLPO, it is highly likely that both mechanisms interact in controlling the expression of vigilance states.

However, the regulation of substates, such as the occurrence of PS, falls within the remit of an ultradian cycle as suggested by the multiple re-occurrence of PS in both human and animals (Aserinsky & Kleitman, 1953; M. Jouvet et al., 1959c). The rebound in the proportion of PS during sleep observed in animals deprived of PS indicates that PS is also homeostatically regulated (D. Jouvet et al., 1964; Sapin et al., 2009). However, these mechanisms are still poorly understood. Structurally, numerous regions have been identified as being involved in the regulation of PS homeostasis as described in subsection 1.3.1. Recent studies revealed that the hypothalamic preoptic area neurons projecting towards the SLD gradually become more active during SWS as PS restriction increased the number of calcium peaks (Maurer et al., 2024). Furthermore, inhibiting these neurons reduced the PS rebound (Maurer et al., 2024). While these results suggest that the preoptic area of the hypothalamus is mediating the homeostatic regulation of PS, they also support the notion that PS is highly dependent on SWS.

### 1.3.3 The hypothesized function of sleep

Knowing how vigilance states are regulated however does not yield a clear answer regarding their respective function. Cirelli and Tononi (2008) posit that if sleep were not essential, there should be: 1- animals that do not sleep; 2- animals that do not show a homeostatic rebound of sleep; and 3- that the lack of sleep should not bear any consequences. Extensive ethological and comparative studies report that sleep-like states and substates occur in all species observed (Blumberg et al., 2020; Campbell & Tobler, 1984; Cirelli & Tononi, 2008). The mortality resulting

from prolonged sleep deprivation and the homeostatic regulation of sleep observed in all species where such elements have been measured suggests that sleep indeed fulfils one or many functions (Cirelli & Tononi, 2008; Rechtschaffen et al., 2002).

The beneficial effects of sleep however should not be treated as a function as they might be a corollary effect, as warned by Bodisz (Bodizs, 2021) using the example of the function of eating: “A good meal could result in better mood, but no physiologist would claim that a function of eating is mood restoration.”

The hypotheses surrounding the function(s) of sleep can be subdivided into several groups or meta-theories: the ecological hypothesis, sleep as a rest, sleep as neuronal detoxification and restitution, sleep for learning and memory consolidation, sleep for network maintenance, and the immunology theory of sleep (Bodizs, 2021), with each theory exploring both sleep as a unified state and the effects of each substate. While these are all theories, they are not mutually exclusive and it is still entirely plausible that sleep as a whole or substates of sleep exert one, some, or all of these functions.

The ecological hypothesis differs from the other theories as it suggests that sleep does not directly exert any vital function. Observations of changes in behaviour following a nycthemeral rhythms in species from the animal, fungal and plant kingdoms suggest the recurrence of a dormant state allowing for behaviour occurring during the day to be highly specialised and therefore more efficient (J. M. Siegel, 2009). A dormant state, such as the one only measured in animal, occurring at a time with high predator activity might allow a higher chance of survival by concentrating vital wake-associated activities at time when the predatory pressure is at its lowest.

Most of the other theories involve some restorative properties of sleep. The function of sleep could be energy and metabolism-related, as suggested by the decrease in adenosine levels during sleep (Porkka-Heiskanen & Kalinchuk, 2011). However, if this was due to an overall decrease in neuronal activity during SWS, then PS and the associated wake-like activity would be highly inefficient. Sleep has also been shown to facilitate the clearance of the cerebrospinal fluid and removal of toxins from the cerebral environment, supporting the neuronal detoxification

hypothesis (Xie et al., 2013). Other reports show that sleep deprivation negatively impacts inflammation, while sleep increases immune responses (Bodizs, 2021). Thus, it appears that should sleep, as a whole, have a function, it might be the restoration of other bodily functions.

Sleep is also heavily implicated in memory processes. Numerous evidence points towards a beneficial impact of sleep on procedural and declarative memory in human and rodents (Jenkins & Dallenbach, 1924; Rolls et al., 2011; Walker et al., 2002). Periods of sleep have also been shown to enhance cortical remodelling processes following monocular deprivation in kitten at a greater rate than the same period of wake in darkness (Frank et al., 2001), and there is growing evidence supporting structural synaptic changes occurring during sleep (de Vivo et al., 2017; Vyazovskiy et al., 2017). However, the key method commonly used to explore the effects of sleep is sleep deprivation. While it has been shown that several methods exist to sleep-deprive a subject (Milinski et al., 2021), the wake experience induced is either different from physiological wake, if not somewhat stressful. The latter might suggest that the effects measured following sleep deprivation are more reflective of increased stress rather than lack of sleep, as longer period of sleep deprivation requires stronger stimulation to prevent the animals to fall asleep with increased handling and poking (Rial et al., 2007).

Tied to the homeostatic regulation of sleep, the synaptic homeostasis hypothesis (SHY) proposed by Tononi and Cirelli (Tononi & Cirelli, 2003, 2006) suggests that the recovery effects of sleep benefit network maintenance. According to this view active learning happens during wake, leading to a net increase in synaptic weights. The synaptic homeostasis hypothesis posits that during sleep, to counterbalance the effects of wake, there is a process promoting a net decrease in synaptic weight, which allows a better signal to noise ratio upon waking and a lowering of the energy demands of the network (Tononi & Cirelli, 2003, 2006). Synaptic downscaling has been observed in the cortex during SWS (Vyazovskiy et al., 2008), again supporting the notion that slow wave activity have a strong role in the network maintenance

occurring during sleep (Bartram et al., 2017).

The homeostatic aspect of SWA occurring during sleep supports the idea that sleep-related electrophysiological events promote structural and/or synaptic changes in the brain ultimately leading to the positive change in learning, memory, and metabolism observed as a result of sleep. Mechanistically, the bistability of both the neurons and the network observed during slow waves has been shown to mediate these structural changes *in vitro* (Bartram et al., 2017) and *in vivo* (González-Rueda et al., 2018). During slow waves, spike-timing dependent plasticity occurs which leads to the selective weakening of synapses (Bartram et al., 2017; González-Rueda et al., 2018). These restorative properties of slow waves are thought to be mediated by glutamatergic neurotransmission (Vyazovskiy et al., 2017). Therefore, the occurrence of slow waves at a local or global level observed during either prolonged wakefulness or during repetitive, low-attention tasks (Fisher et al., 2016; Vyazovskiy et al., 2011b) might result in an increased capacity to maintain a state of wakefulness (Vyazovskiy & Tobler, 2012).

The interest surrounding slow wave-associated processes, however, casts a shadow on other sleep-related events if not entire substates. Indeed, the decrease in attention inevitably associated with increased slow wave activity during wakefulness can potentially explain the deficit in learning and memory elicited by sleep deprivation (Andrillon et al., 2021; Vyazovskiy & Tobler, 2012). However, global hypotheses integrating all sleep-related events and substates may provide a better understanding of how an interplay between vigilance states might lead to a net brain-wide recovery. The association of spindles, slow waves, and ultra-fast hippocampal sharp wave ripples is thought to actively participate in the information transfer to long-term storage from short-term memory associated structures like the hippocampus (Buzsáki, 1998). This idea is supported by observations suggesting that PS might be involved in the regulation and maintenance of short-term memory processes through synaptic downscaling mechanisms (Grosmark et al., 2012). On the other hand, however, sleep spindles have also been associated with the maintaining of

sleep states (Dang-Vu et al., 2010; Loomis et al., 1935). Spindles therefore have been directly associated with both memory consolidation and the integrity of sleep, suggesting that the occurrence of a disconnected state is relevant for the overall maintenance of the brain networks (Fernandez & Lüthi, 2020).

The overall function of sleep is still heavily debated. The features of sleep-like slow waves can point to mechanisms involved in network maintenance, but whether these mechanisms alone can elucidate the function of a state is still largely unclear. The function of PS for instance is still largely unclear. The activation of hippocampal neurons during PS suggests a potential role in contextual memory consolidation, as supported by the observation that suppression of hippocampal theta rhythm during PS impairs contextual memory consolidation (Boyce et al., 2016). With the occurrence of strong and vivid experiences like dreams PS is also thought to be involved in emotional memory processes (Luppi et al., 2022), although such a connexion remains to be investigated (A. M. Stephan et al., 2021), and emotional memory-related processes have been shown to occur throughout sleep (Deliens et al., 2014).

Whether sleep as whole has any function at all can also be argued. Indeed, it is plausible that asking for the function of sleep is like asking for the function of wake. The need for disconnection is as a puzzling question, as is the need for connection. During wake, as the functions of specific behaviours are studied rather than wake as a whole with its plethora of different yet concomitant processes, only the analysis of specific sleep-associated behaviours might be relevant.

Here, I have presented slow waves as an event associated with sleep that is proposed to play a role in synaptic plasticity. We also discussed that slow waves are not sleep-bound and strongly involved in the notion of a network disconnected from the environment like in local occurrence of slow waves. Therefore, the alteration of brain oscillations like slow wave activity might result in the occurrence of disconnected states, but also short- and long-term changes in brain processes.

## 1.4 Restoring mental health

### 1.4.1 The relationship between sleep and mental health

As sleep has been shown to have an important influence on the regulation and restoration of cerebral networks, dysregulation of the sleep/wake cycle is commonly associated with mental health disorders such as affective disorders (Melo et al., 2017; Wehr et al., 1979). A large majority of people affected by major depressive disorder and other mental-health related conditions often report sleep disruptions, insomnia, or hypersomnia which are thought to, in turn, worsen the condition of the patient (Geoffroy et al., 2018; Wichniak et al., 2017). The comorbidity between sleep disorders and mental-health disorders potentially results from alterations in the neuromodulatory systems, including dysregulation of the 5-HT system.

The European Sleep Research Society recently recommended the use of cognitive behavioural therapy (CBT) as a first line of treatment against insomnia and depressive disorders (Riemann et al., 2017). CBT is a therapeutic approach with multiple methodologies including improvement of sleep hygiene, sleep-restriction protocols, conditioning of sleep, relaxation, and education around sleep (Gavriloff et al., 2022).

Paradoxically, a surprising alternative therapeutic approach to depressive symptoms is sleep deprivation (Pflug & Tolle, 1971; Wirz-Justice & Van den Hoofdakker, 1999). Indeed, a meta-analysis of 66 independent studies suggests that an acute antidepressant effect can occur following total and partial sleep deprivation (Boland et al., 2017). The therapeutic properties of sleep deprivation for affective disorders however are not fundamentally strong, as they are reverted by the subsequent night of sleep, and paradoxical, as partial sleep disruptions are detrimental to mental health (Boland et al., 2017).

Classical pharmacological approaches for the treatment of depressive disorders target monoaminergic neurotransmission, for example selective serotonin reuptake inhibitors (SSRI) or monoamine oxidase inhibitors (MAOI) (Wichniak et al., 2017). Both MAOIs and SSRIs are thought to act by increasing the levels of monoamines

in the brain, with SSRI increasing 5-HT concentration levels specifically and used as a first line treatment with non-immediate effects and requiring a course treatment (Baker et al., 1992; Sharp et al., 1997). It has been shown that SSRI, like other antidepressants, promotes neural plasticity, this is thought to be the mechanism underlying their therapeutic effects (Maya Vetencourt et al., 2008). To achieve a maximal therapeutic effect, these drugs are sometimes used jointly with other compounds. Indeed, using 5-HT<sub>2A</sub> receptor antagonists have been shown to augment the effects of SSRIs (Boothman et al., 2006), potentially due to the silencing of a negative 5-HT<sub>2</sub>- feedback loop.

These types of drugs are however not without an impact on sleep and wake states (Wichniak et al., 2017). One of the main reported effects is a prominent increase of PS latency, with an overall decrease of PS expression following SSRIs and MAOIs (Wichniak et al., 2017), in line with the current understanding that PS generation relies on a low to absent monoaminergic release. Other antidepressants have mixed effects on sleep and wake states (Wichniak et al., 2017) with some reports suggesting a worsening of sleep and increased rates of acute and chronic insomnia (Doghramji & Jangro, 2016), while others report an improvement of sleep (Thompson, 2002).

With the restorative properties of sleep, and considering the proposed role of sleep in network renormalisation, altering monoaminergic neurotransmission could lead either to wake-promotion or sleep enhancement, either of which could contribute to an improvement of depressive symptoms. While the effects of antidepressants on sleep and wake states are well studied and documented, the prospect that, in turn, sleep might mediate antidepressant mechanisms has received little attention.

### 1.4.2 The association of psychedelics and sleep

It has been shown that sleep and wake states are heavily influenced by one another, as altering the content of wake can lead to a lighter or deeper subsequent sleep (Fisher et al., 2016; Milinski et al., 2021; Vyazovskiy et al., 2011a, 2011b). These experimental alterations of wake are a valid option to study any hypothetical



functions of sleep (Bodizs, 2021). As presented in section 1.1, it is entirely possible to alter the content of wake through pharmacological means. In humans, caffeine, tobacco and alcohol usage are commonly used and socially accepted drugs in western culture, but cocaine, opioids, and cannabinoids are also in recreational use. All of these compounds have different effects on sleep and wake, often corresponding to their recreational effects (Frase & Nissen, 2022). Caffeine induces wake-promoting effects (Snyder, 1985), stimulants like cocaine and tobacco induce insomnia and sleep disruption, whereas opioids and alcohol tend to increase SWS (Frase & Nissen, 2022).

Despite the link between sleep and mental health disorders, and the potential therapeutic effects of 5-HT psychedelics as well as the dream-like experiences they might produce, only a few studies have looked comprehensively at the effects of psychedelics on sleep-wake states and *vice versa*. Reports suggest that in humans, midday administration of 20 mg of Ayahuasca, equivalent to 1 mg/kg DMT, leads to prolonged PS latency (Barbanoj et al., 2008). In comparison, morning administration of 0.2 mg/kg psilocybin has been shown to acutely increase sleep fragmentation and to delay PS onset, without any long term change on sleep EEG features like SWA (Dudysova et al., 2020). An equivalent study conducted on mice with the administration of 2 mg/kg psilocin at light onset found similar results (Thomas et al., 2022). These results are also in line with the effects of antidepressant like SSRI.

Comparing the effects of classical psychedelics to SSRI and MAOI antidepressants might, as with comparing sleep between species, help in understanding the mechanisms underlying the antidepressant effects. As the suppression of PS is a common effect of antidepressants (Wichniak et al., 2017), at the very least measuring the expression of vigilance states could provide a proxy readout of antidepressant effects, thus offering useful practical information. Jointly, studying how sleep and brain states respond to psychedelics could arguably provide important new insights into the function of specific brain states. The anaesthetic ketamine, while

not a classical psychedelic, is also being investigated as a potential novel rapid-acting antidepressant as a single dose of ketamine has also been reported to induce anti-depressant effects (E. E. Lee et al., 2015).

While the underlying mechanisms are still unclear, results suggest that these effects are achieved through glutamatergic neurotransmission and it was proposed that EEG slow waves could play a role (W. C. Duncan et al., 2019; Duncan et al., 2013). It is therefore highly likely that, instead of a direct pharmacological antidepressant effect, the fast-acting antidepressants like ketamine or classical psychedelics might promote the occurrence of pre-existing brain mechanisms like slow waves, processes known to be involved in the restorative aspect of sleeps. However, as there are few studies conducted on the effect of psychedelics on sleep, the analysis of the state induced by psychedelic substances in comparison to spontaneous sleep and wake has received little attention.

### 1.4.3 **Vigilance states are an overlooked yet essential notion**

The discovery and description of vigilance states through the occurrence of their hallmark behavioural and electrophysiological features has been outlined in section 1.2. Wake, SWS and PS can all be usually separated based on their traditional defining criteria. Moreover, while local occurrence of sleep has been measured during wake, there are numerous lines of evidence that each of these states are regulated at a global level through an extensive neuromodulatory network (Fort et al., 2009). However, the analysis of the brain using these markers is often confined to sleep research and not applied to other neuroscientific fields like psychedelic research. This is an important omission. Indeed, it is well known that psychedelic intoxication induces stereotypical changes in behaviour, as seen with the head-twitch response in rodents (Halberstadt et al., 2020) which has been shown to be highly susceptible to the circadian rhythm (Moser & Redfern, 1985).

Furthermore, psychedelic injections induce alterations of a given EEG frequency band (Murray et al., 2022; Riga et al., 2016, 2018; Vejmola et al., 2021). Thus,

neglecting the notion of sleep and wake cycle, encompassing with it circadian rhythmicity, might lead to strong bias. Trying to categorise the psychedelic state as a specific brain state with set features as was done for SWS and PS decades ago might be beneficial and help understanding the wider effects of psychedelics.

In other words, rather than investigating the effects of psychedelics on sleep-wake states, we hypothesise that psychedelics induce a unique, distinct state of vigilance, that can be best understood in comparison to conventional vigilance states but is not merely their extension. As there is currently no consensus on what exactly psychedelics are by focussing on their pharmacology or on the subjective alteration of wake experience (Lepow et al., 2023; Nichols et al., 2022; O'Donnell et al., 2023; Zanos et al., 2018), properly defining the psychedelic state will offer a new and fresh perspective into understanding their underlying neurobiology.

The mechanism underlying therapeutic effect of classical psychedelics is not known but might involve increased neural plasticity mediated by the activation of 5-HT<sub>2A</sub> receptors and which may only be clinically effective when associated with psychotherapy (Rucker et al., 2018). The psychedelic experience itself might be relevant too, as positive experience, mindset, and environment are thought to impact the outcome of the treatment (Carhart-Harris et al., 2018b). The context of all these studies in humans is clearly associated with wake experience. However, most studies conducted on rodents completely disregard the brain state of the animal. It is entirely plausible that the effects on EEG reported so far are entirely determined or modified by the occurrence of SWS or PS episodes.

There is currently a great deal of interest in identifying the mechanism(s) through which psychedelics exert their therapeutic effects as it might lead to the making of a designer drug which has the potential to provide better treatment for a high number of mental health disorders. However, identifying potential mechanisms and brain signals as markers of the psychedelic state while disregarding the brain states might be senseless. Psychedelics indeed have been shown to promote wakefulness. However, this implies that over the period of time immediately following the injection, while

all animals treated with psychedelics were awake, the animals treated with vehicle had periods of sleep (Thomas et al., 2022). Thus, analysing the acute EEG effects of psychedelics and comparing them with vehicle, while disregarding the state of the animal, inevitably will lead to an erroneous interpretation of psychedelic effects. Crucially, it is possible to argue that it is the state during which the psychedelic effects occur that ultimately matters, rather than changes in brain activity, which merely reflect the state.

To this end, we argue that it is essential to consider both brain activity and behaviour when studying the immediate effects of psychedelics. Behavioural states can be scored and identified based on the movement of the animal and can refine the analysis by segregating wake and sleep (Vejmola et al., 2021). However, as psychedelics have been associated with decreased motility (Geyer & Light, 1979), such approaches might leave out key elements corresponding to the psychedelic effect as occurring during an inactive phase. Therefore, other readouts of the state of the animal, including brain activity are also necessary.

## 1.5 Hypothesis and aims

Numerous lines of evidence suggest that the 5-HT system is heavily implicated in the regulation of physiological vigilance states. With psychedelics inducing profound changes in wake experience while being non-specific agonists of numerous wake-promoting 5-HT receptors, it is entirely plausible to consider 5-HT not only as a neuromodulator capable of promoting change in the *quantity* of states by promoting wakefulness and repressing PS, but also capable of altering the *quality* of states by inducing altered states of vigilance. This psychedelic-induced state of vigilance could therefore foster a cerebral environment beneficial to the occurrence of elevated plasticity or other phenomena which are ultimately responsible for their beneficial effects.

The main hypothesis of this DPhil project is that the activation of serotonergic receptors by psychedelics acutely produces an altered brain state identifiable by

electrophysiological and behavioural features. The main goal is therefore to define and explore the vigilance state induced by psychedelics in laboratory mice. To this end, we will rely on 5-MeO-DMT, a short-acting classical psychedelic. As opposed to LSD and psilocybin, 5-MeO-DMT has a higher affinity for the 5-HT<sub>1A</sub> receptor compared to the 5-HT<sub>2A</sub> with effects dissipating within one hour as opposed to the several hours of altered behaviour and brain activity following LSD and psilocybin (Ermakova et al., 2022; Halberstadt et al., 2012). One advantage of using this compound is its short-lasting effects, which overcome the potential confound of delayed, indirect effects of the drug arising as a result of sleep-wake disruption (Thomas et al., 2022).

This thesis has been constructed based on three distinct aims:

1. The characterisation of the state induced by 5-MeO-DMT in terms of its electrophysiological and behavioural features (Chapter 3).
2. The investigation of the alteration in wake quality instilled by 5-MeO-DMT in terms of cortical and autonomic arousal, and sensory perception (Chapter 4 & Chapter 5).
3. The identification of the regulatory mechanisms responsible for the occurrence of the identified 5-MeO-DMT-induced state and its features (Chapter 6).

Chapter 3 addresses the first objective and aims to characterise the acute changes in vigilance states induced by 5-MeO-DMT in mice. There, with the measurement of EEG and EMG recordings, as well as behavioural responses to the administration of 5-MeO-DMT, an altered state was successfully identified and its features described. Chapter 4 addresses the alteration of arousal induced by 5-MeO-DMT, participating in the further characterisation of the psychedelic-induced altered state and offering novel metrics capable of measuring the dynamics of 5-MeO-DMT actions. Chapter 5 addresses the alteration of perception induced by 5-MeO-DMT, further suggesting alteration in the quality of wake. Chapter 6 presents potential

mechanisms responsible for the induction of the reported effects using behavioural and pharmacological approaches.

# 2

## General methods

### Contents

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|            |                                                  |           |
|------------|--------------------------------------------------|-----------|
| <b>2.1</b> | <b>Animal husbandry . . . . .</b>                | <b>46</b> |
| <b>2.2</b> | <b>Surgeries . . . . .</b>                       | <b>47</b> |
| 2.2.1      | Pre-operative procedures . . . . .               | 47        |
| 2.2.2      | Intracranial implants . . . . .                  | 48        |
| 2.2.3      | Intracortical implants . . . . .                 | 50        |
| 2.2.4      | Post-operative care . . . . .                    | 51        |
| <b>2.3</b> | <b>Injections . . . . .</b>                      | <b>52</b> |
| <b>2.4</b> | <b>Sleep deprivation . . . . .</b>               | <b>53</b> |
| <b>2.5</b> | <b>Data acquisition and processing . . . . .</b> | <b>54</b> |
| 2.5.1      | Data acquisition . . . . .                       | 54        |
| 2.5.2      | Off-line analysis . . . . .                      | 54        |
| 2.5.3      | Sleep scoring . . . . .                          | 55        |
| 2.5.4      | Analysis parameters . . . . .                    | 56        |
| 2.5.5      | Statistical analysis . . . . .                   | 57        |

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This chapter details those experimental procedures, materials and analysis methods which are common among the projects reported in this thesis. Here are covered the origin and husbandry of animals, key experimental procedures such as surgeries, drug/vehicle injections and sleep deprivation, as well as details of data acquisition, processing and analysis. The “surgeries” section is subdivided into subparts detailing preoperative care, the different types of implants, and animal welfare provision such as post-operative monitoring. The “data acquisition

and processing” section is subdivided into subparts giving details about the data acquisition, the different analysis pipelines, manual sleep scoring rules, and statistical analyses. Each of the following results chapters of this thesis also contains a specific methods section giving a summary of the procedures and analysis methods used, as well as more details on the methods should they vary from the ones presented in this chapter.

All procedures were performed under a UK Home Office Project License and conformed to the Animals (Scientific Procedures) Act 1986 under personal and project licences granted by the United Kingdom Home Office. Ethical approval was provided by the Ethical Review Panel at the University of Oxford. Animal holding and experimentation was undertaken at the Biomedical Sciences Building (BSB), University of Oxford.

## 2.1 Animal husbandry

Adult male C57BL/6J mice were acquired either in-house from BSB from the C57BL/6JOLA-HsdOxuni lineage ( $n = 16$ ), or from Charles River ( $n = 47$ ), for a total of 63 animals aged from 9 to 15 weeks old. All sample sizes were selected based on statistical power calculation made on large effect size for a significance level of 0.05, based on results obtained at a similar dose (Martin et al., 1985; Moser & Redfern, 1985). Upon receipt, at 8 weeks-old, the mice were kept single-housed in ventilated cages in level 1 of the BSB and were left undisturbed for at least a week prior to transferring them to another level of BSB for experimental procedures. Only male mice were used in this project to reduce unknown confounding variables. We however would like to acknowledge the need to perform the experiments presented in this thesis on female mice as the literature is extremely sparse on that aspect. Furthermore, this specie was selected as an extension of previous work (Thomas et al., 2022).

During the experiments, the mice were kept single-housed in individual Plexiglas cages (20.3cm x 32cm x 35cm) placed inside ventilated, sound-attenuated Faraday chambers (Campden Instruments, Loughborough, UK), under a 12 – 12 hour



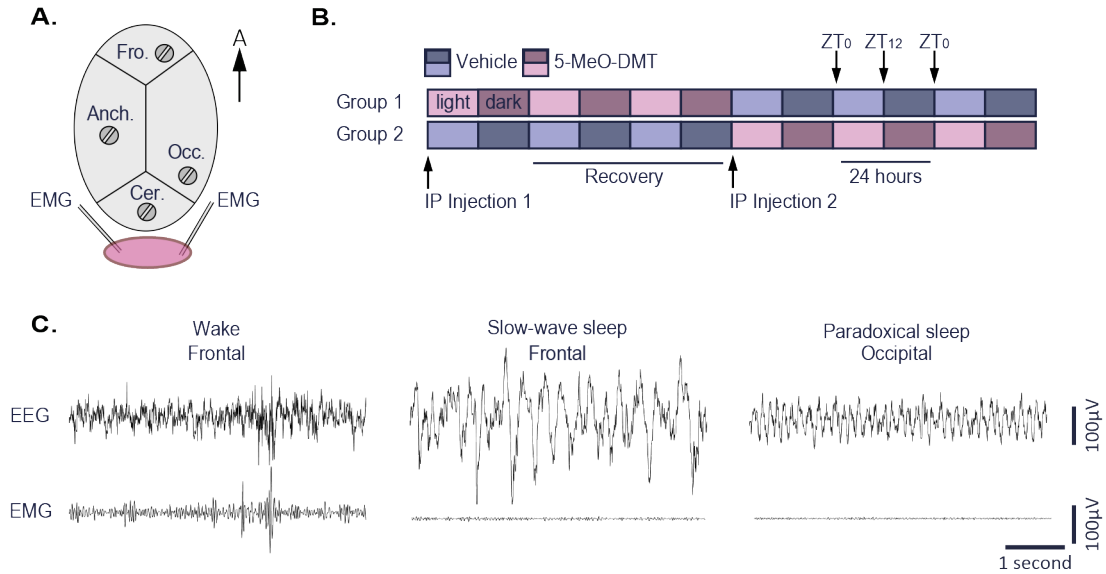
light-dark cycle (9 am ON - 9 pm OFF unless otherwise mentioned) in the Sleep and Circadian Neuroscience Unit in BSB. The recording room was maintained at  $22 \pm 1$  °C and  $50 \pm 20\%$  humidity. Food and water were provided ad libitum throughout the experiment (unless otherwise mentioned). The back of the cages was removable to fix a running wheel. All chambers had an overhead webcam fitted to a hole in the ceiling for continuous remote monitoring. In the following chapters, “home cage” refers to the Plexiglas cages in the Faraday chambers (EMC shielded environmental cabinet, Campden Instrument).

Before the start of the experimental procedures, the animals were left undisturbed for 3 to 5 days in their home cages. At the end of the procedures, most animals ( $n = 48$ ) were euthanized by exposing to rising concentration of CO<sub>2</sub> and cervical dislocation. The subset of animals with intracortical implants ( $n = 14$ ) received terminal anaesthesia followed by transcardiac perfusion and brain collection for histological processing. Results from histological processing are available in the annexes of their respective chapters. The remaining animals either died during the recovery from surgery from a non-procedure related stomach ulcer ( $n = 1$ ), or during terminal anaesthesia followed by cervical dislocation ( $n = 2$ ).

## **2.2 Surgeries**

### **2.2.1 Pre-operative procedures**

Throughout the surgical procedures, mice were kept in their cages on BSB level 1. Three days before the surgery, the mice were habituated to strawberry jelly and mashed food pellet in preparation for the administration of post-operative analgesia and welfare checks. On the day of the experiment, the mice were moved to the Sleep and Circadian Neuroscience Unit suite where they were weighed and prepared for the surgery.



**Figure 2.1: General experimental plan.** **A.** Default EEG implantation surgeries. Fro = Frontal. Occ = Occipital. Cer = Cerebellum. Anch = Anchor screw. The arrow points towards the anterior part of the animal. The EMG are implanted in the nuchal muscle. **B.** Default injection protocol. All animals are subdivided into two groups. Group 1 received 5-MeO-DMT first, group 2 the vehicle. Most animals received their injection at light onset, unless mentioned otherwise. 72 hours after the first injection, the animals received a second injection of the compound they did not receive. **C.** Representative EEG and EMG signal for all vigilance states. Wake is associated with EEG signal of fast frequency and low amplitude, with high EMG activity. SWS is defined by large slow-waves of high amplitude in the frontal part of the brain, with a low-to-absent muscle activity. In PS, the occipital EEG shows an increased theta activity due to the proximity of the electrode to the hippocampus, and a flat EMG due to muscle atonia.

### 2.2.2 Intracranial implants

Intracranial implants were performed based on established protocols for device implantation in mice (Fisher et al., 2016; Krone et al., 2021). All mice subjected to in vivo electrophysiological recordings ( $n = 36$ ) were implanted intracranially with EEG screws for signals acquisition, together with EMG wires in the nuchal muscles (Figure 2.1.A). While the procedure remains the same, some animals were implanted according to different coordinates to accommodate other implantats such as cannulas or intracortical electrodes. The stereotaxic coordinates used in these animals are detailed in their respective chapters.

Prior to surgery, custom-made head stages were made using stainless steel screws (0.86 mm diameter, InterFoxus Ltd, Cambridge, UK), stainless steel wires, eight-

pin and 90° connectors (Pinnacle Technology Inc, Kansas, USA, model 8415-SM). Connections were soldered and sealed with Simplex Rapid dental acrylic cement (Associated Dental Products Ltd, Swindon, UK). The head stages were composed of at least 3 screws and two wires with naked, twisted ends, connected to the eight-pin connectors. All head stages were tested before the implantation and sterilised for aseptic surgery using 70% ethanol.

Mice were anaesthetised in an induction chamber with 4% isoflurane at an oxygen flow rate of 4 L/min until righting and pedal reflexes were lost. Mice were then transferred to a heat pad and anaesthesia delivered through a nose mask at 2% isoflurane with an oxygen flow rate of 2 L/min. The head of the mouse was shaved at the location of the implant, and Metacam® (meloxicam, 5 mg/kg, Boehringer Ingelheim Ltd., Bracknell, UK) and Vetergesic® (buprenorphine, 0.1 mg/kg, Sogeval UK Ltd., York, UK) were used for analgesia via subcutaneous injections. 5 mL/kg per expected hour of surgery of 0.9% sterile saline was injected subcutaneously to compensate for fluid loss. The mice were then transferred to a stereotactic surgery frame (David Kopf Instruments, California, USA; Model 900 Small Animal Stereotaxic Instrument) resting on a cover sheet on top of a body-temperature controlled heat pad, and fitted with a rectal probe to maintain the core body temperature at around 36°C. The head of the animal was adjusted in a teeth-holder and fixed with ear bars. The isoflurane concentration was adjusted based on the breathing rate (target rate 50-80 breaths per min) and was kept stable using an isoflurane concentration between 0.6- 1.2% at a flow rate of 1.5 L/min throughout the surgery. Regular anaesthesia checks were performed every 15-30 minutes by a surgery assistant. Viscotears Liquid Gel® (Alcon Laboratories Limited, Hemel Hempstead, UK) was applied for eye protection before the implantation site was thoroughly cleaned with povidone-iodine and ethanol.

A midline incision was performed with a scalpel blade from the level of the eyes to approximately 1.5 cm caudal to the ears, and the skin flaps retracted using surgical retractors or bulldog clips. Connective tissue was then removed, and the skull surface

cleaned with saline before being scratched with the tip of the scalpel blade. The skull was then etched with 37% phosphoric acid etching gel, cleaned with a cotton tip and rinsed with saline. Together, the scratching and etch gel allowed for the cement to have a better attachment to the skull by creating a more irregular surface. The level of the skull was calibrated to be within a 0.2 mm variation between the dorso-ventral coordinates for Bregma and Lambda. Implantation sites were marked on the skull using a needle-tip with ink attached to the stereotactic arm.

In total, 3 coordinates were marked for an EEG electrodes: Frontal (AP: +2.0 mm; ML: +2.0 mm), Occipital (AP: -3.5; ML: +2.5), and Reference screw (2 mm behind Lambda, ML: 0 mm) positions. An additional anchor screw location was marked (AP: -3.5; ML: -2.5) if no other intracranial or intracortical implantations were performed to balance the head stage. A surgical drill with a 0.6 mm diameter carbon steel burr (InterFocus Ltd, Linton, UK) was used to prepare skull holes for the epidural insertion of the stainless-steel screws. The exposed dura at the bottom of the holes was kept moist by applying sterile saline to the holes immediately after drilling. All EEG screws and anchor screws were carefully implanted in the designated holes and fixed with dental cement SuperBond® (Prestige Dental Products Ltd, Bradford, UK). Two stainless steel wires were implanted bilaterally into muscle pockets, which were created with the tips of sharp forceps planted into the nuchal muscles.

Implants and wires were then secured with dental cement. At the end of the procedure, the skin in the neck was sutured, 5 mL/kg per expected hour of surgery 0.9% sterile saline was injected subcutaneously to compensate for fluid loss if the first injection did not cover the time of the entire surgery. The animal was removed from the stereotaxic frame, weighed, and placed in a recovery box at 27 °C until locomotor activity was observed.

### 2.2.3 Intracortical implants

In some animals ( $n = 14$ ) intracortical electrodes (16-channel laminar probe NeuroNexus Technologies Inc., Ann Arbor, MI, USA; model: A1x16-3mm-100-703-Z16)

or cannulae were implanted. The coordinates of each implant are detailed in their respective chapters. The laminar probes were stained with an orange-red fluorescent membrane stain Dil® (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; Thermo Fisher Scientific, Waltham, MA, USA) shortly before the surgery and immediately protected from light exposure to minimize bleaching. A concentration of approximately 20 mg/ml Dil dissolved in 50/50% acetone/methanol was found to be most suitable to ensure a reliable demarcation of the electrode tract without excessive diffusion of the stain into the surrounding tissue.

Implantation of intracortical elements was usually performed before the EEG screws were secured with dental cement. First, under microscopic control, the bone around the implantation site was thinned in a circle (1 mm to 1.5mm diameter) with a surgical drill with a 0.6 mm diameter carbon steel burr (InterFocus Ltd, Linton, UK). This technique allowed for better protection of the cortex around the electrode site. Moments before inserting the probe, the remaining skull bone at the centre of the circle was lifted using a needle and the area covered in saline. Removal of the bone facilitated the removal of the attached dura. The laminar probe was attached to a ZIF-Clip® 16 channel headstage (Tucker-Davis Technologies Inc., Alachua, FL, USA), fixed to a stereotaxic arm (Model 900 Small Animal Stereotaxic Instrument, David Kopf Instruments, California, USA) and carefully lowered over the implantation site. Once the bone was removed, the arm was lowered so that the implant rested on the pial surface and then inserted into the tissue at the right depth under stereotactic and microscopic control. Immediately after implantation, the site was sealed with the silicone elastomer Kwik-Sil® (World Precision Instruments Inc., Sarasota, FL, USA). The electrode shaft was then cemented to the neighbouring implants and the electrode was grounded and referenced to the cerebellum screw.

## 2.2.4 Post-operative care

Animals were monitored closely in the recovery box until the recovery of locomotor activity. They were then returned to a ventilated cage with soft bedding, strawberry

jelly with oral Metacam, mashed food pellet as well as diverse seeds and whole food pellets. The cage was left on a heat mat for at least an hour under constant monitoring before being returned to the holding room. During the first 3 days, mice were checked twice a day using a standardised scoring sheet to assess the health status and evaluate the need for post-operative care. They received a daily dose of strawberry jelly with oral Metacam for post-operative analgesia. If required, the animals received an intraperitoneal injection of Vetergesic and Metacam. They were then monitored daily for the following 3 days and were considered recovered if no analgesic was delivered. Mice then moved into their home cages in the Sleep and Circadian Neuroscience Unit for habituation.

## 2.3 Injections

To investigate the effects of psychedelics on vigilance states, but also on behaviour, arousal and evoked responses, we used 5-MeO-DMT supplied by Beckley (BPL-003, Beckley Psytech, UK). 5-MeO-DMT is a Class A drug under the Misuse of Drugs Act 1971 and Schedule 1 of the Misuse of Drugs Regulations of 2001. Utilisation and storage of 5-MeO-DMT was conducted according to the controlled drugs license from the Home Office bestowed upon co-supervisors. 5-MeO-DMT was selected for its strong, fast-acting properties inducing dream-like experience in humans and behavioural changes in rodents within 5 minutes of administration. Following an IP injection in mice, 5-MeO-DMT has a maximum concentration in plasma reached after 5 minutes and a terminal half-life of 12-19 minutes (Shen et al., 2009). On the day of the injection, 1 mg of 5-MeO-DMT was diluted into 1 mL of 0.9% sterile saline and syringes containing either enough for an injection of 5 mg/kg 5-MeO-DMT or the vehicle (5 mL/kg sterile 0.9% saline) were prepared. The dose of 5-MeO-DMT was selected based on previous work reporting increased head twitch response at this dose without the occurrence of tremors and other strong behavioural shifts (Martin et al., 1985; Moser & Redfern, 1985).

After one day of baseline electrophysiological recording, the mice were subjected to a counterbalanced, crossover design whereby they received a first intraperitoneal injection at light onset (ZT0) with either 5-MeO-DMT or vehicle and were then left undisturbed unless mentioned otherwise (Figure 2.1.B). 72 hours following the first injections, the mice received an intraperitoneal injection of the substance they did not originally receive (Figure 2.1.B). The large majority injected animals ( $n = 55$ ) followed the above injection protocol. A similar version with the injections at a different ZT was performed for another group of animals ( $n = 8$ ). A small number of animals did not receive IP injections but intracortical injections instead ( $n = 4$ ). All variations are detailed in their respective chapters.

## 2.4 Sleep deprivation

To investigate the influence of sleep homeostasis on 5-MeO-DMT-induced changes in brain activity, or on attention, or to keep the animals awake during the recordings, mice ( $n = 20$ ) were subjected to sleep-deprivation procedures from ZT0 for a given time detailed in their respective chapters. At light onset, the chambers were open and nesting material removed from the home cages. Removing the nesting material introduces changes to the environment that keeps the animals awake for around 30 minutes, during which the animals explored their environment or exhibited nest-building behaviour using the bedding of the cage. Repeated nest-building behaviour was immediately followed by manual introduction of novel objects in the cage. Novel objects included small wooden blocks, folded gloves, and various toys, which were added to the cage or replaced as required. This procedure is known to increase homeostatic sleep pressure and subsequent EEG slow-wave activity during subsequent sleep and relies on the natural exploratory behaviour of mice (Huber et al., 2007; McKillop et al., 2018; Thomas et al., 2022). The mice were continuously monitored by the experimenters, and novel objects were introduced into the environment as soon as the animal was immobile or as sleep-like slow waves

became visible on the EEG.

## 2.5 Data acquisition and processing

### 2.5.1 Data acquisition

Electrophysiological signals were acquired using a 128-channel Neurophysiology Recording System (Tucker-Davis Technologies Inc., Florida, USA). Mice were connected to the system via custom made cables connecting to their head stage with enough length to leave them free to roam around their entire cages. All signals were amplified (PZ5 NeuroDigitizer preamplifier, Tucker-Davis Technologies Inc., Florida, USA) and filtered online between 0.1 Hz and 100 Hz for EEG, LFP, and EMG signals and saved at a sampling rate of 305 Hz. Extracellular neuronal activity was recorded at a sampling rate of 25 kHz and filtered between 300 Hz and 5 kHz. The threshold was set differently for each channel and for each animal, based on visual estimation of the level of noise present in the recordings. 46 samples at 0.04 ms of interval surrounding the crossing of the threshold were stored, consisting of 0.48 ms before and 1.36 ms after.

Some experiments required running wheel or auditory analysis. Details concerning these methods are given in Chapter 3 and Chapter 4. Regarding signal acquisition, running wheel signals and auditory stimulations were stored at a sampling rate of 25 kHz. Running wheel signals were not continuous recordings of the wheel but timestamp detection of the wheel bars crossing an infrared beam. The elevated 25 kHz sampling rate allows for a timestamp with a high decimal number thus preventing missing events due to duplicated values.

### 2.5.2 Off-line analysis

Two separate working pipelines were created on MATLAB (The MathWorks Inc, Natick, Massachusetts, USA, version v2023a) for this project, following the notion of reusability between experiments. First, the Signal Processing Pipeline was



constructed as a general pipeline to process the signal from the recording up to obtention of the Fast-Fourier transform. Then, the "Psychedelic pipeline" was created to fit the analysis requirements of this project with every function being flexible enough to be reused by other experiments without alteration through a set file-naming nomenclature and a fixed file structure. To allow for a cross-experiment reusability of scripts, an "experimental design" script was created to link each experiment to a metadata file containing all of the information needed to analyse the experiment, from file location, to recording dates of interests, frequencies, channels, exact procedure times and more. This allowed the creation of one single metadata .mat file containing all of the information that would be in a format fitting the entirety of the two pipelines and allow for the same, consistent and reproducible automated analysis for all experiments, together with proper annotation. This greatly increased the data processing speed and promoted the collaboration within the lab, while producing draft figures for easy visualisation.

For the signal processing pipelines, raw signals were exported from the experimental recording computers and backed up on a server and an external hard drive. The signals were also stored on a working hard drive of the computer. They were then resampled at a sampling rate of 256 Hz using custom made scripts. These signals were then filtered with a zero-phase Chebyshev type filter (0.5 Hz to 120 Hz for EEG, 4 Hz or 10 Hz to 100 Hz for EMG) and converted into the European Data Format with a joint MATLAB-Python script. While sleep signals were then used for sleep scoring, other signals acquired were processed through different functions of the signal processing pipeline, detailed in their own chapters. This includes the extraction of stimuli, as well as running wheel data.

### 2.5.3 Sleep scoring

Vigilance states were manually scored using the signals in European Data Format using SleepSign (Kissei Comtec, Nagano, Japan). The signals were divided in epochs of 4 seconds. Each epoch was scored based on visual inspections of frontal,

occipital and EMG signals (Figure 2.1.C). If recorded, LFP signals were also used. Epochs with recording artefacts due to gross movements, chewing or external electrostatic noise were assigned to the respective vigilance state but not included in the electrophysiological analysis. Wake was scored in epoch containing high EMG activity. SWS was scored in epochs containing low EMG and visual slow activity (0.5 Hz to 4 Hz) of high amplitude in the frontal or in both frontal and occipital derivations. PS was scored when the occipital signal was mostly defined by theta activity (7 Hz to 12.5 Hz) with low EMG. Epochs containing a mixed frequencies of lower amplitude and high EMG between two sleep epochs (SWS – SWS or PS – SWS) were scored as movement, unless 5 or more consecutive similar epochs were scored, in which case it was classified as wake. After sleep scoring, the power spectra of the signal were computed using a fast Fourier transform routine (Hanning window) with a 0.25 Hz resolution and exported in the frequency range between 0 and 120 Hz for spectral analysis.

#### 2.5.4 Analysis parameters

Fast Fourier transform signals were stored into .mat files using a custom MATLAB script containing the spectrum of each epoch and the associated vigilance state. The spectral extraction script also corrected the epoch mistakenly scored as movement when they should have been scored as wake, and *vice versa*. Indeed, as established in 2.5.3, movements were scored if 4 consecutive epochs or less showing high EMG activity were surrounded by sleep epoch. Scoring error involving the presence of too many movement epochs or too few wakes were uncommon but, if made, can easily be missed at correction. The spectral extraction script detected the length of consecutive movement or wake epochs. If 5 or more consecutive movement or movements artefacts were detected, the script replaced them as wake and wake artefact epochs. If less than 5 consecutive epochs of wake and wake artefact were detected, the script replaced them as movement and movement artefact epochs.

The output files were processed through the “psychedelic pipeline.” The pipeline consisted of 6 different independent parts. The use of each part depended on each experiment, and more specific versions will be given in their respective chapters. All experiments passed through the first two parts.

The first part calculated the mean spectra per state of vigilance for given time frame. The spectra were also used to determine the time course of the EEG/LFP activity in a given frequency range. The activity was calculated as the area under the average spectral curve of the frequency range of interest at a given time. This calculation was conducted across the desired time period at a resolution of one data point every 2 seconds. The time frames of interest were a period of 30 minutes immediately after the injection, and a period of 60 minutes, occurring 1 hour following the injection. Thus, measuring the immediate acute effects of the injection, and the potential effects occurring more than 1 hour after the injection.

The second part computed the sleep architecture. Episode onsets were calculated based on the first occurrence of an uninterrupted episode lasting longer than 1 minutes for SWS and 32 seconds in the case of PS to accommodate for their transition period. Sleep stability was calculated based on the amount of movement episodes per consolidated SWS episode. The following parts cover specific experiments and will be detailed in their respective chapters.

### 2.5.5 Statistical analysis

Statistical analyses were computed using Prism (GraphPad Software, Boston, Massachusetts USA, version 10.2.0 (392)). All data sets were tested for normality with the Shapiro-Wilk test, and sphericity tested with Spearman’s rank correlation test. If all assumptions were met, a parametric test was conducted, otherwise its non-parametric equivalent was selected for  $\alpha = 0.05$ . Each chapter will detail which tests were used. On figures, a significant difference is plotted as a straight black or coloured line in spectrogram analyses, as plotting the difference for each data bin would make the figure less readable. For all other figures, significance

levels are indicated with black asterisks: \* for  $p \in [0.05; 0.01]$ , \*\* for  $p \in ]0.01; 0.001]$ , \*\*\* for  $p \in ]0.001; 0.0001]$ , \*\*\*\* for  $p < 0.0001$ . Figures were generated using Prism. show group means with SEM for parametric data or median and interquartile ranges for non-parametric data, unless mentioned otherwise in the figure legend, such as for plotting representative raw signals of one animal. The legend of each figure provides a reminder of the meaning of each symbols including significance level. The appendices of this thesis contains tables with the detailed results of the statistical analyses conducted per chapter.

# 3

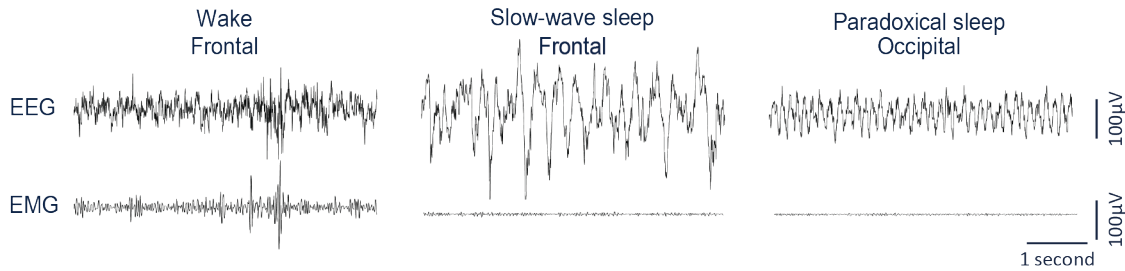
## The effects of 5-MeO-DMT on vigilance states and brain activity

### Contents

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|            |                                                                                    |           |
|------------|------------------------------------------------------------------------------------|-----------|
| <b>3.1</b> | <b>Introduction</b>                                                                | <b>60</b> |
| 3.1.1      | Defining states of vigilance                                                       | 60        |
| 3.1.2      | The alteration of states by psychedelics                                           | 62        |
| 3.1.3      | Experimental aims                                                                  | 65        |
| <b>3.2</b> | <b>Methods</b>                                                                     | <b>66</b> |
| 3.2.1      | Animal husbandry                                                                   | 66        |
| 3.2.2      | Surgery                                                                            | 66        |
| 3.2.3      | Injections                                                                         | 67        |
| 3.2.4      | Behaviour monitoring                                                               | 68        |
| 3.2.5      | Analysis                                                                           | 69        |
| <b>3.3</b> | <b>Results</b>                                                                     | <b>70</b> |
| 3.3.1      | 5-MeO-DMT injection induces changes in waking behaviour                            | 70        |
| 3.3.2      | 5-MeO-DMT injection results in acute, global and local alteration of brain signals | 74        |
| 3.3.3      | 5-MeO-DMT alters sleep architecture but not sleep signals                          | 79        |
| <b>3.4</b> | <b>Discussion</b>                                                                  | <b>83</b> |
| 3.4.1      | The changes in waking behaviour                                                    | 83        |
| 3.4.2      | The changes in goal-oriented behaviour                                             | 85        |
| 3.4.3      | A paradoxical wake state                                                           | 86        |
| 3.4.4      | 5-MeO-DMT and sleep                                                                | 87        |
| 3.4.5      | Further analyses                                                                   | 88        |
| 3.4.6      | Conclusion                                                                         | 90        |

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**Figure 3.1: The electrophysiology of vigilance states.** Representative EEG traces of one animal acquired during an experiment presented in this chapter. EEG and EMG activity are used to score Vigilance states in rodents. Wake is associated with EEG signal of fast frequency and low amplitude, with high EMG activity. SWS is defined by large slow-waves of high amplitude in the frontal part of the brain, with a low-to-absent muscle activity. In PS, the occipital EEG shows an increased theta activity due to the proximity of the electrode to the hippocampus, and a flat EMG due to muscle atonia.

## 3.1 Introduction

### 3.1.1 Defining states of vigilance

The notion of vigilance states can be applied to many different genera in the animal kingdom through behavioural and electrophysiological criteria. Indeed, in all species studied so far were observed a behaviourally active state, wake, and a state in opposition, sleep (Blumberg et al., 2020; Cirelli & Tononi, 2008; Piéron, 1913; Tobler & Borbély, 1985). As described in Chapter 1, electrophysiological studies of sleep and wake states allowed for a classification of vigilance states, substates, and their corresponding regulation from the 1930s onwards (Fort et al., 2009). And while there might undoubtedly be as many different expressions of vigilance states as there are species, the robust definition of states and their regulations defined from the 1930s allows cross-species comparison as seen in Chapter 1 (Blumberg et al., 2020; Fort et al., 2009; Tobler & Borbély, 1985).

Three vigilance states have been described in mammals using behavioural and electrophysiological criteria: wakefulness and two main stages of sleep; slow wave sleep (SWS) or non-rapid eye movement sleep (NREM), and paradoxical sleep

(PS) or REM sleep (Aserinsky & Kleitman, 1953; Dement, 1958; M. Jouvét & Michel, 1958; M. Jouvét et al., 1959c).

Wakefulness is typically a behaviourally active state, as reflected by movement, accompanied by high EMG activity and EEG activity of low amplitude signals rich in theta (5 – 10 Hz) and higher frequencies (Yamagata et al., 2021) (Figure 3.1). Sleep, however, is marked by an immobility of the animal and reduced behavioural responsiveness to external stimuli. Sleep can be further divided into two substates: slow wave sleep (SWS) and paradoxical sleep (PS).

As EEG activity is typically correlated with arousal and behaviour, the main electrophysiological feature of SWS in rodents is the occurrence of EEG slow oscillation (0.5 Hz to 4 Hz) of high amplitude (Steriade et al., 1993b; Wilson & Groves, 1981) (Figure 3.1). Slow waves are thought to originate from a bistability of the network, following an oscillatory activity pattern between depolarised ON- and hyperpolarised OFF-periods (Nir et al., 2011; Vyazovskiy et al., 2009b). The occurrence of slow waves, as described in Chapter 1, could illustrate a default cortical state with a generative pattern from the cortical layer V, entrained by cortico-thalamic networks (David et al., 2013; M. Jouvét & Michel, 1958; M. Jouvét et al., 1959c; Krone et al., 2021; Steriade & McCarley, 2013).

PS, as opposed to SWS, is defined by wake-like EEG dominated by theta-frequency activity (6 Hz to 10 Hz). This origin of this rhythm can be traced to the medial septal area which greatly projects towards the hippocampus (Boyce et al., 2016; Mitchell et al., 1982). The wake-like cortical activity comes with a complete muscle atonia interrupted in animals by transient twitches of the limbs, ears and whiskers and accompanied by rapid eye movements in both humans and animals (Aserinsky & Kleitman, 1953; Dement, 1958; M. Jouvét et al., 1959c) (Figure 3.1).

Wake and sleep architecture refers to the notion of state repartition over a given time period, relevant to the species studied. The architecture of states changes markedly with species. Humans, as a reference, show cycles between SWS and PS every one hour and 40 minutes during the night period (Aserinsky & Kleitman, 1953). Rodents, and more specifically mice, typically used in sleep studies are nocturnal

animals in laboratory conditions. They therefore produce more episodes of SWS and PS in the light period compared to the dark period. As argued in Chapter 1, sleep in mice is heavily fragmented. SWS bouts are about 10-minutes long, while PS episodes last no more than 5 minutes, with an average of 30 transitions from SWS to PS in 24 hours in adult mice (Campbell & Tobler, 1984; Franken et al., 1998; Valatx & Bugat, 1974). These transitions between states can happen at even higher rates, as mice have been found to have, on average, 1660 state transitions per day (Soltani et al., 2019).

Despite these robust definitions, the boundaries between vigilance states are not well defined. Hallmark features of states are not bound to said states, as suggested, for example, by the observation of local slow waves occurring during wakefulness. Indeed, sleep deprivation is known to increase slow activity in the EEG even in behaviourally awake animals (Borbély et al., 1984; Vyazovskiy et al., 2011b). Repetitive activity, such as running on a running wheel, has been associated with increased slow wave activity during wakefulness (Fisher et al., 2016). Local incursion of these sleep-like patterns of activity are not without consequences, as they have been shown to negatively correlate with performance and cognitive processes during wakefulness (Andrillon et al., 2021; Nir et al., 2017; Vyazovskiy et al., 2011b). These observations suggest that vigilance states are not homogeneous and that it is highly probable that processes commonly associated with sleep can occur during an altered wake state comprised of sleep-like brain signals. Given that sleep affects plasticity processes, potentially providing a restorative function, the occurrence of sleep-like brain signals might also promote the occurrence of said restorative functions during wake states.

### 3.1.2 The alteration of states by psychedelics

Psychedelics are a fascinating class of drugs known to profoundly alter sensory and behavioural experiences in humans, most commonly through altered 5-HT



neurotransmission (Barrett et al., 2015; Barsuglia et al., 2018; Nichols et al., 2022; Nutt et al., 2020). They were first used as a tool to better understand psychiatric disorders such as schizophrenia (Osmond, 1957), before being shown to have potential therapeutic properties when used as an adjunct to psychotherapy in a range of psychiatric disorders (Barrett et al., 2020; Carhart-Harris & Goodwin, 2017; Rucker et al., 2018). In humans, the administration of psychedelics can induce transient dissociated states of vigilance, confusion or thought disorder. and profound psychoactive effect on perception (Barrett et al., 2015; Barsuglia et al., 2018; Carhart-Harris et al., 2016b; Griffiths et al., 2016). In mice, the potency of a psychedelic can be correlated with head-twitch responses (HTR), defined as brisk movements of the head. They are often used as a marker of the acute effects of the drug with HTRs being the main behavioural feature of an alleged psychedelic state in rodents (Halberstadt et al., 2020). It has been shown that higher doses of psychedelics can also induce strong behavioural changes such as hind limb abduction and head weaving, but also in rare cases backward walking, straub tail, tremors, low body posture, hyperactivity and decrease in body temperature, behaviours associated with the serotonin syndrome (SS) in humans, resulting from an over-stimulation of serotonergic receptors (Haberzettl et al., 2013; Sun-Edelstein et al., 2008). Using EEG recordings showed that psychedelics promote broadband desynchronisation (Murray et al., 2022; Vejmola et al., 2021).

As argued in Chapter 1, the therapeutic properties of psychedelics are still poorly understood. Clinical trials suggest that psychedelics can be effectively used to treat a wide range of mental illnesses and seem to have a long term effect close to classical antidepressants like SSRIs and MAOIs but with a positive outcome occurring much faster (Carhart-Harris et al., 2018a; Rucker et al., 2018; Wichniak et al., 2017). These mental illnesses are also commonly associated with sleep disorders (Gavriloff et al., 2022). Albeit small, the literature on sleep and psychedelics, which has been discussed in Chapter 1, showed that psychedelic intoxication leads to an alteration of sleep. In humans, a daytime administration of a capsule of ayahuasca

(1mg/kg of DMT) or psilocybin (0.25 mg/kg) induced an increase in PS latency (Barbanoj et al., 2008; Dudysová et al., 2020). In rodents, administration of 2 mg/kg psilocin also increased PS latency (Thomas et al., 2022). The analysis of electrophysiological markers of sleep following daytime administration of psychedelics in human suggests an elevated EEG power between 15 Hz and 20 Hz during stage 1 and stage 2 (Barbanoj et al., 2008; Dudysová et al., 2020), while slow wave activity was increased but only throughout the first cycle of sleep (Barbanoj et al., 2008). Rodent studies in which the injection was conducted at light onset, however, report that slow frequencies have an elevated EEG activity during wake and PS but not SWS (Thomas et al., 2022). The possibility exists that this acute disruption of states may be essential for the therapeutic effects. However, this disruption is still poorly understood. Taken together, the behavioural and electrophysiological studies with psychedelics point towards the idea that the psychedelic state is indeed associated with changes in global state control, resulting in a shift to an altered state of vigilance.

The characterisation of the psychedelic state as an altered state of vigilance has, surprisingly, received very little attention. Current studies on laboratory rodents mostly separate vigilance states as one behaviourally active and one behaviourally inactive state (Vejmola et al., 2021). A more robust approach to score vigilance states should allow the detailed characterisation of the acute state induced by psychedelic intoxication. A recent study used psilocin, the active, dephosphorylated form of psilocybin known to induce alterations for several hours (Thomas et al., 2022). After sleep scoring method relying on EEG and EMG signals to estimate the vigilance state, this study showed that an injection of psilocin promotes arousal by increasing time spent awake, increasing sleep instability and PS latency as well (Thomas et al., 2022). However, as psilocin is a long-lasting compound, the disruption of sleep states is likely due to the wake-promoting properties resulting from 5-HT activations. The use of shorter lasting compounds such as 5-MeO-DMT can confine the effects on an initial period of wakefulness. Thus, any effects on

subsequent sleep measured would likely be an effect of a disruption of sleep processes occurring during the hypothesised acute state.

### 3.1.3 Experimental aims

The general aim of this chapter is to better characterise a potential “psychedelic state” through the identification of key behavioural and electrophysiological markers. Current features of the behavioural and neurophysiological state induced by psychedelics include HTR, dysregulation of sleep states and EEG desynchronisation. The first part of this chapter presents the acute behavioural changes induced by an injection of 5-MeO-DMT in terms of behavioural psychedelic responses, such as HTR, but also in terms of locomotion and food-driven exploration. The second part of the chapter details the acute effects of 5-MeO-DMT on EEG and LFP recordings and describes potential hallmark electrophysiological features of the psychedelic state. The third part of the results section investigates delayed effects of the injection of 5-MeO-DMT, and therefore of the psychedelic state, on the subsequent sleep architecture and associated brain signals. These results can potentially lead to a better understanding of the transient alterations caused by psychedelics on wake experience, as reported by human users, as well as the potential therapeutic effects of psychedelics.

The experimental aims of this chapter are to:

1. Assess whether 5-MeO-DMT induces acute changes in the waking behaviour of mice.
2. Explore how 5-MeO-DMT affects wake and sleep in terms of electrophysiology.
3. Investigate if the induction of a psychedelic state impacts subsequent sleep architecture and sleep electrophysiology.

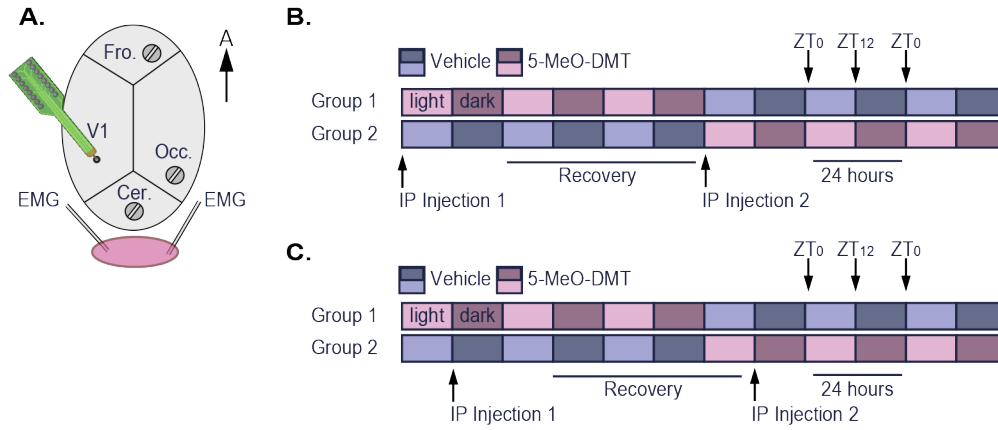
## 3.2 Methods

### 3.2.1 Animal husbandry

Adult male C57BL/6JOlaHsdOxuni mice ( $n = 22$ , Oxford Biomedical Science colony, 10 to 14 weeks old) were kept singly housed in individual Plexiglas cages (20.3 cm x 32 cm x 35 cm), placed inside ventilated sound-attenuated Faraday chambers (Campden Instruments, Loughborough, UK), under a 12-12-hour light-dark cycle (9 am - 9 pm unless otherwise mentioned). The recording room was maintained at  $22 \pm 1$  °C and  $50 \pm 20$  % humidity. Food and water were provided ad libitum throughout the experiment. All procedures were performed under a UK Home Office Project License and conformed to the Animals (Scientific Procedures) Act 1986.

### 3.2.2 Surgery

To investigate the effects of 5-MeO-DMT on sleep architecture and wake behaviour, a subset of the animals used in this chapter ( $n = 10$ ) were implanted with EEG screws and EMG wires as described in Chapter 2. To monitor further intra-cortical activity, a subset of these animals ( $n = 6$ ) was implanted with laminar probes (A1x16-3mm-100-703-Z16, NeuroNexus) after a 1 x 2 mm craniotomy was made using a high-speed drill above the primary visual cortex (-3.4 mm anteroposterior (AP), -2 mediolateral (ML)), sealed and protected by a two-component silicone gel (KwikSil; World Precision Instruments) (Figure 3.2.A). The signals were referenced to the cerebellum screw. The visual cortex was targeted to investigate possible changes in cortical activity in one of the primary sensory areas, which could provide insights into neural correlates of the well-known alteration of visual experience associated with the psychedelic experience in humans (Nichols & Nichols, 2008). The signals of the laminar probe of 2 animals had to be discarded for technical reasons.



**Figure 3.2: Experimental plan of Chapter 3.** **A.** Representation of surgical implant localisation. Fro = Frontal. Occ = Occipital. Cer = Cerebellum. V1 = Primary visual cortex. The arrow points towards the anterior part of the animal. The EMG are implanted in the nuchal muscle. **B-C.** Injection protocols. All animals are subdivided into two groups. Group 1 received 5-MeO-DMT first, group 2 the vehicle. All implanted animals received their injection at light (B) onset to analyse the effect of 5-MeO-DMT on the subsequent sleep episodes ( $n = 14$ ) or dark onset (C; ZT = 12) to analyse awake behaviour ( $n = 8$ ). 72 hours after the first injection, all animals received a second injection of the compound they did not receive.

### 3.2.3 Injections

After one day of baseline behavioural monitoring, all injected mice ( $n = 22$ ) underwent a pharmacological study in a pseudorandom and counterbalanced, crossover design consisting of an injection of either vehicle (5mL/kg saline) or 5 mg/kg 5-MeO-DMT (1 mg/ml saline, Beckley Psytech) on day 1, followed by two days of recovery, then a second injection of the other solution on day 4, after which they were allowed two further days of recovery (Figure 3.2.B). Each solution was prepared fresh before the experiment and administered by intraperitoneal injection at light onset (ZT0) for the implanted animals, and for a subgroup of unimplanted animals ( $n = 4$ ) as detailed in Chapter 2. For the behavioural experiments ( $n = 8$ ), the injections were conducted at dark onset to maximise the chance to observe spontaneous active behaviour (ZT = 12, Figure 3.2.C).

### 3.2.4 Behaviour monitoring

To assess the effects of 5-MeO-DMT on HTR, 4 mice were recorded in their home cages for 1 hour after the injection time. HTR were scored by observation of a brisk, paroxysmal rotation of the head, not associated with grooming or exploratory behaviour (Halberstadt et al., 2020).

To assess the effects of the compound on exploratory behaviour, 4 animals received a bowl of 12 sugar pellets (Sucrose Tab/Fruit Punch 14MG, 5TUT - 1811324, TestDiet). On day 0, the filled bowl was introduced without prior habituation 10 minutes after dark onset (ZT12). The injection protocol began on day 1. The bowl of sugar pellets was placed on the cage floor 10 minutes after the injection. Video recording of the behaving animal was acquired for one hour following the injection and analysed offline. Bowl interaction and pellet eating behaviours were manually scored by a blind experimenter. A bowl interaction was recorded as close-range exploratory behaviour targeted at the bowl and was typically made with the whiskers and snout.

To assess the effects of 5-MeO-DMT on locomotor activity, 4 animals were given access to running wheels (Campden Instruments, Loughborough, UK, wheel diameter 14 cm, 38 bars spaced 1.11 cm apart (inclusive of bars)), a behavioural assay relying on the tendency of mice to spontaneously run in such an apparatus. Installation of the setup and habituation were performed based on established protocols (Milinski et al., 2021). The wheels were installed in the home cages of the animals for at least four weeks prior to the recordings. Each wheel was fitted with a digital counter (Campden Instruments) detecting bars passing the infra-red emitter/receiver at a 24.4141 kHz recording frequency using the signal acquisition system described in Chapter 2. The digital counter output was a 5 V TTL pulse edge detector which sent a signal in the acquisition system with timestamp. The timestamps were analysed offline in Matlab (The MathWorks Inc., Natick, MA, USA). With each wheel having a diameter of 14 cm and 38 bars making a complete revolution, speed was first calculated in rotations per second, before being converted to cm/s. The injection protocol began on day 1. The animals

were placed in the running wheels 10 minutes following each injection and were left otherwise undisturbed to assess spontaneous activity. Video recordings and running wheel activity were acquired for one hour following the injection and analysed offline.

### 3.2.5 Analysis

Electrophysiological data were processed offline using MATLAB (The Math Works Inc., USA) as described in section 2.5.2. Vigilance states were assessed using SleepSign (Kissei Comtec, Nagano, Japan) as described in section 2.5.3. EEG delta and theta power were calculated as area under the FFT curve obtained, as described in section 2.5.4, between 0.5 – 4 Hz for delta and 6 – 10 Hz for theta. The detection of LFP slow waves and corresponding neuronal signals from laminar probes implanted in the primary visual cortex were extracted through the detection of peaks and troughs in the filtered signal. The corresponding wave amplitude was calculated and only the waves whose maximum amplitude was above the standard deviation of all detected waves were selected.

Data were processed offline using MATLAB (The Math Works Inc., USA), and analysed using MATLAB and Prism (GraphPad Software, Boston, Massachusetts USA, version 10.2.0 (392)). All data were tested for normality prior to analysis using a Shapiro-Wilk test. Non-parametric tests were used unless all conditions for a parametric test were met. All statistics were conducted on GraphPad Prism. All comparisons were made within-subjects unless mentioned otherwise. Simple linear regressions were computed on the time course of HTR and compared using an F test. To compare means between two groups, paired t-tests were conducted or another non-parametric equivalent. When three conditions were compared, a repeated-measure (RM) one-way ANOVA with Tukey's post hoc tests was performed, or a Friedman test and a post-hoc Dunn's multiple comparisons test as a non-parametric equivalent. Mixed-effects (ME) analysis and RM-two-way ANOVAs were performed when comparing multiple variables with Tukey's post hoc tests.

Bonferroni correction was not applied for post hoc comparisons of spectral data because ANOVAs consisting of 119 EEG spectral bins would result in Bonferroni comparisons being too conservative and reduce statistical power, thus Fisher's LSD tests were used for post hoc analysis (Achermann and Borbély 1998). The Greenhouse-Geisser (GG) correction was applied when sphericity of the data was not found. The results of the statistical analyses are detailed in Appendix A.

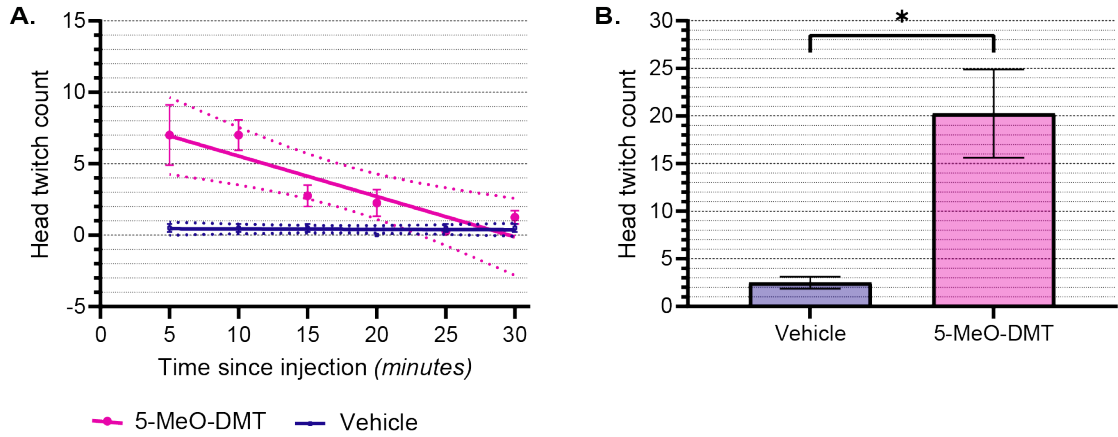
### 3.3 Results

#### 3.3.1 5-MeO-DMT injection induces changes in waking behaviour

To verify that the dose and drug we acquired for this project had an effect, we first measured HTR in four animals. Indeed, an expected effect of psychedelics in mice is to induce marked head twitches and, even though DMT-class psychedelics induce less head twitches compared to other drugs (Halberstadt et al., 2020), the measurement of the HTR is a first step that would allow us to make a first assessment of the psychedelic potency of this drug at a particular dose. The dose of 5 mg/kg of 5-MeO-DMT was selected as it is an effective, medium dose based on a previous study in the literature (Halberstadt et al., 2020). The mice were kept in their home cages, but their nest was removed to force them to exhibit spontaneous behaviours typically observed during waking such as exploration, grooming, or nest-building.

The injection of 5-MeO-DMT resulted in notable changes in the spontaneous behaviour of all mice. The treated animals exhibited somewhat altered behaviour characterised by exploration of the bedding in their immediate surroundings with their forelimbs, whiskers and snout, and less grooming, and less rearing behaviour in the hour following the injection. The injection of 5-MeO-DMT also resulted in a transient increase in head twitches, an expected change following an injection of psychedelics (F test on slopes comparing vehicle and psychedelic condition following simple linear regressions  $F_{1,4} = 18.52$ ;  $p < 0.01$ ) (Figure 3.3.A). This transient increase led to a significant difference in the total HTR between the two conditions





**Figure 3.3: The analysis of head twitches.** **A.** Time course of head twitch response 30 minutes following an injection of 5-MeO-DMT or vehicle at light onset, and corresponding fitted linear regressions. The vehicle line was not significantly different from 0 ( $p = 0.80$ ), but the 5-MeO-DMT line was significantly different from 0 ( $p = 0.011$ ). The slope of the two lines are significantly different ( $p < 0.01$ ). **B.** Total counts of HTR (mean + SEM). A paired t-test revealed a significant difference between the two groups ( $*p < 0.05$ ).  $N = 4$  for all panels. See Table A.1 for statistical analyses.

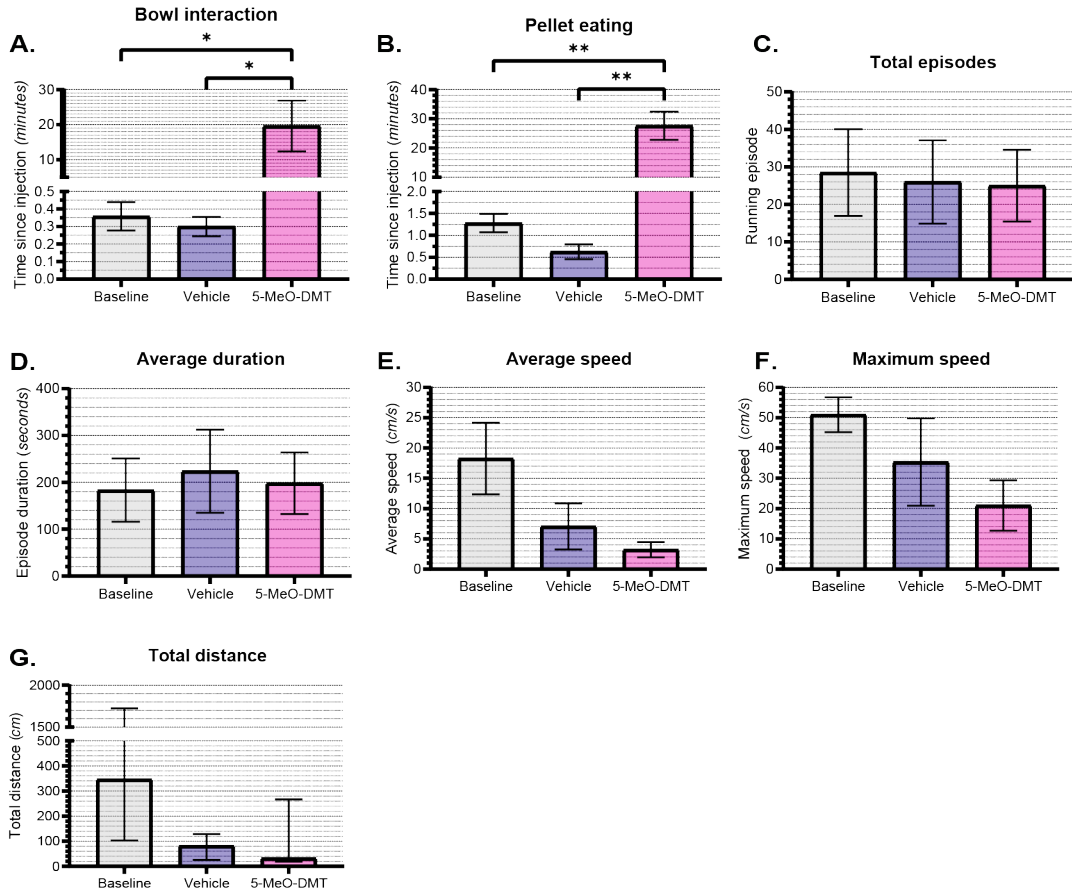
(t-test,  $t_3 = 3.96$ ;  $p < 0.05$ ) (Figure 3.3.B).

The possibility remains that the injection of 5-MeO-DMT merely induced the onset of the “serotonin syndrome” (SS) characterised by behavioural deficits or locomotor abnormalities (Haberzettl et al., 2013). Although we never observed abnormal behaviours such as hyperactivity, flat body posture, catalepsy, hind limb abduction, or full body tremors, we decided to perform two additional experiments in non-implanted mice which were provided access to the running wheels or given sugar pellets. The running wheel experiment was performed to assess body posture and voluntary locomotor activity. The sugar pellet experiment was performed to assess environment awareness and interaction. These experiments were performed at dark onset, when laboratory mice are typically awake spontaneously, and would be expected to display normal, species-specific behaviours. We therefore assessed whether these interventions would reveal abnormalities in locomotor, sensory and motivational aspects of wakefulness in the presence of 5-MeO-DMT.

As noted in the previous cohort, the mice remained active, and their behaviour was dominated by exploration, grooming and nesting following either drug or vehicle injection. All these different behaviours were invariably observed in all animals, including both controls and 5-MeO-DMT injected animals. After an injection of 5-MeO-DMT, all mice showed HTR, and exploration behaviour targeted at the bedding in their immediate surroundings. As the nests were kept in the home cages on this occasion some mice spent most of the recording time in the nest, visibly awake as shown by an active behaviour.

To characterise the sensory and motivational components of wakefulness, we presented the animals with a plastic cup of sugar pellets 10 minutes after the injection, corresponding to the time when the effects of the 5-MeO-DMT have fully developed (Halberstadt et al., 2020). We observed that invariably the vehicle group approached and explored or interacted with the cup and its content  $0.3 \pm 0.06$  minutes ( $18 \pm 3.34$  seconds) after it was presented, retrieving the first pellet, on average, after  $0.63 \pm 0.17$  minutes ( $37.5 \pm 10.15$  seconds) (Figure 3.4.A-B). In contrast, after an injection of 5-MeO-DMT, the animals started exploring the cup after a significantly longer time ( $19.62 \pm 7.27$  minutes; RM-one-way ANOVA, effect of condition  $F_{2,6} = 7.07$   $p = 0.026$ ; Tukey's post hoc  $p < 0.05$  against baseline and vehicle respectively), and only engaged with the cup and attempted to eat the pellets after  $27.65 \pm 4.88$  minutes (RM-one-way ANOVA  $F_{2,6} = 28.95$   $p < 0.001$ ; Tukey's post hoc  $p < 0.01$  against both baseline and vehicle) (Figure 3.4.A-B).

To characterise the spontaneous locomotor activity, another group of animals had free access to running wheels. To make sure that the animals did not spend their recording time in their nest, all mice were placed inside their running wheel 10 minutes after the injection but were then left free to run or leave the wheel. On average, there was no statistically significant difference between 5-MeO-DMT and vehicle condition, with the same amount of running bouts (RM-one-way ANOVA,  $F_{2,6} = 2.854$ ;  $p = 0.13$ ) (Figure 3.4.C) or bouts duration ( $F_{2,6} = 2.169$ ;  $p = 0.20$ ) (Figure 3.4.D).



**Figure 3.4: The effects of 5-MeO-DMT on behaviour.** A-B. Total time in minutes from injection to exploration of the sugar pellet bowl (A.), or eating the sugar pellets (B.). C-D. Total number of episodes of running (C.) and their average duration (D.) in seconds during 1 hour from dark onset or after the injection of vehicle or 5-MeO-DMT. E-G. Average (E.) and maximal running speed (F.), as well as total distance ran (G.), by the animal on average during 1 hour from dark onset or after the injection. All data are mean + SEM except for G. Total distance ran median + IQRs. (\* $p < 0.05$ ; \*\* $p < 0.01$ ).  $N = 4$  for all panels. See Table A.2 for statistical analyses.

It is however possible that the injection *per se* changed the behaviour of the animals. The average running speed per episode was found not to be significantly different during 5-MeO-DMT ( $3.21 \pm 1.24$  cm/s) compared to baseline ( $18.27 \pm 5.90$  cm/s) despite a numerically lower speed in the 5-MeO-DMT condition (RM-one-way ANOVA,  $F_{2,6} = 2.81$ ,  $p = 0.14$ ) (Figure 3.4.E). It was also noted that, even though non-significant (RM-one-way ANOVA,  $F_{2,6} = 1.81$ ;  $p = 0.24$ ), the animals tended to have a faster maximum speed in the baseline condition ( $51 \pm 5.77$  cm/s) compared to their maximum speed following an injection of 5-MeO-DMT ( $21.03 \pm 8.30$  cm/s)

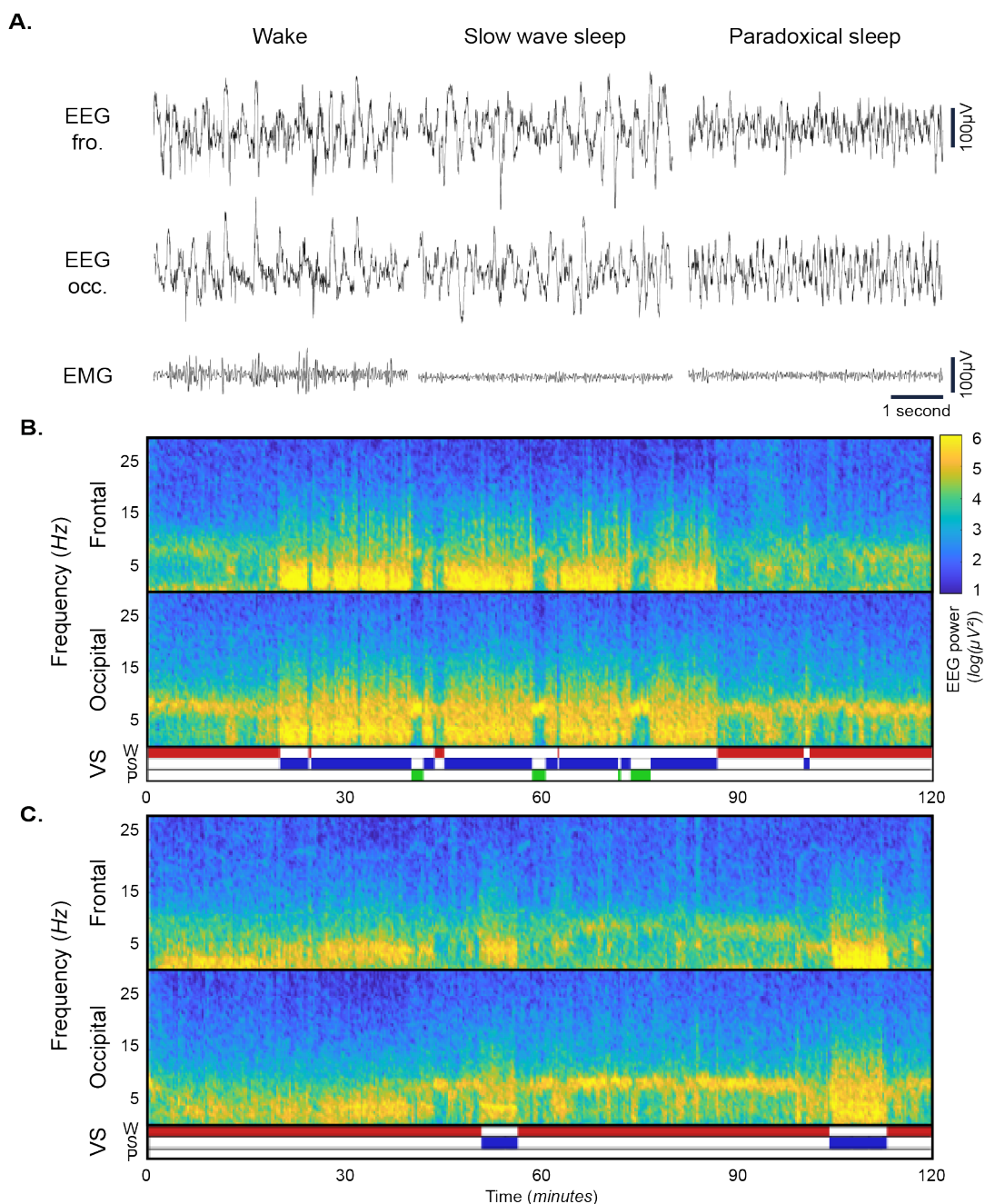
(Figure 3.4.F). This ultimately leads to animals tending to run a lower distance after an injection of 5-MeO-DMT ( $106.3 \pm 78.81$  cm) compared to baseline ( $725.4 \pm 476.7$  cm) (Friedman test,  $F_{2,6} = 4.50$ ,  $p = 0.12$ ; Figure 3.4.G).

### 3.3.2 5-MeO-DMT injection results in acute, global and local alteration of brain signals

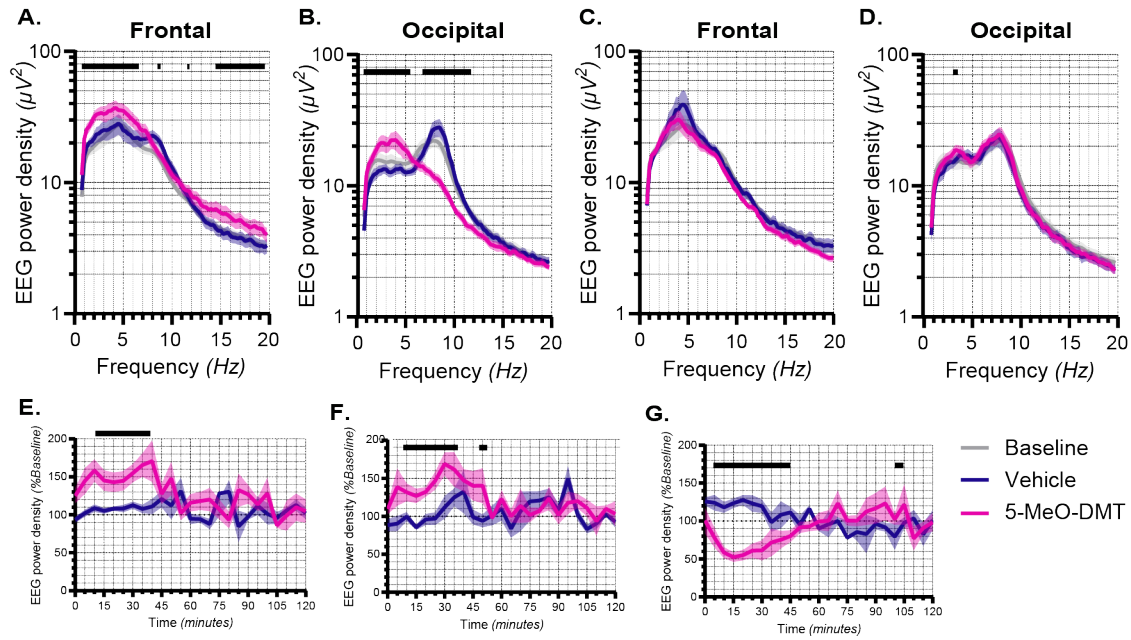
The previous results showed that the dose of 5-MeO-DMT used was sufficient to elicit alteration in behaviour. We therefore expected to observe an acute alteration in brain signals following an injection of 5-MeO-DMT. Surgeries were performed on a separate group of animals ( $n=4$ ) to implant intracranial screws to measure EEG activity, concomitant with EMG activity from the nuchal muscle. A separate group of mice ( $n=6$ ) were also implanted with intra-cortical probes in the primary visual cortex. The intra-cortical electrodes of 2 animals broke during the experiment and so only their EEG and EMG data were obtained.

We first focussed on EEG signals which are traditionally used for determining vigilance states. A striking observation was that following 5-MeO-DMT injection in all mice, EEG slow-wave activity, normally characteristic of SWS, was accompanied by EMG signals typical of the awake state (Figure 3.5.A). Visual observations of the mice after injection of 5-MeO-DMT during this experiment suggested that they were unequivocally awake, demonstrating normal exploratory behaviour, grooming, and walking, while brain activity in both the frontal and occipital derivations was characteristic of SWS (Figure 3.5.A).

A spectral analysis comparing the power of EEG frequencies following the different injections shows that, after an injection of vehicle, the subsequent episodes of waking are associated with strong EEG activity in the theta frequency band in the occipital derivation; SWS is associated with high slow wave activity in both derivations; and PS is associated with a marked theta activity, especially in the occipital derivation (Figure 3.5.B). After an injection of 5-MeO-DMT, however, the



**Figure 3.5: Representative effects of 5-MeO-DMT injections on brain activity**  
**A.** Representative raw traces from the EEG frontal (fro.), occipital (occ.) and EMG derivations following a 5-MeO-DMT injection for wakefulness, sleep and PS over 5 seconds of recordings in one animal. **B.** Representative power spectra of the same animal for the frontal and occipital derivations, with the vigilance states (VR, W = Waking, S = Slow-wave sleep, P = Paradoxical sleep) for 120 minutes following a vehicle injection. **C.** Representative power spectra of one animal for the frontal and occipital derivations, with the vigilance states (VS, W = Waking, S = Slow-wave sleep, P = Paradoxical sleep) for 120 minutes following a 5-MeO-DMT injection.



**Figure 3.6: The alteration of EEG power by 5-MeO-DMT.** A-B. Mean EEG power during the first 30 minutes of wakefulness immediately after an injections of 5-MeO-DMT or vehicle or averaged during 24 hours of wake epochs for baseline in the frontal (A.) and occipital derivation (B.). C-D. Mean and SEM of the EEG power over 2 hours, during wakefulness occurring 1 hour after an injections of 5-MeO-DMT or vehicle or averaged during 24 hours of wake epochs for baseline in the frontal (C.) and occipital (D.) derivations. E-G. Time course of EEG delta activity in the frontal (E.) and occipital (F.) derivations and theta activity in the occipital derivation (G) for 2 hours following an injection of 5-MeO-DMT or vehicle. The horizontal lines denote a significant result at the given frequency bins between 5-MeO-DMT and vehicle. Results shown as mean + SEM. N = 10 for all panels. See Table A.3 & Table A.4 for statistical analyses.

following waking episode shows an increased EEG activity in the delta frequency band in both derivations, with a disappearance of theta-activity. These activity patterns were present for around 45 minutes. Past this time, the activity pattern on both derivations showed a marked activity in the theta frequency range similar to that of vehicle (Figure 3.5.C).

An analysis of the power spectrum for the first 30 minutes of waking following the injection of 5-MeO-DMT, revealed a marked increase of the frontal EEG delta activity (1.25 Hz to 7 Hz) (ME analysis, frequency\*condition:  $F_{152,1060} = 4.25$ ,  $p < 0.0001$ , Fisher's post hoc  $p < 0.05$ ) (Figure 3.6.A) as well as in the occipital derivation (1 Hz to 5.5 Hz); and a significant decrease of the occipital

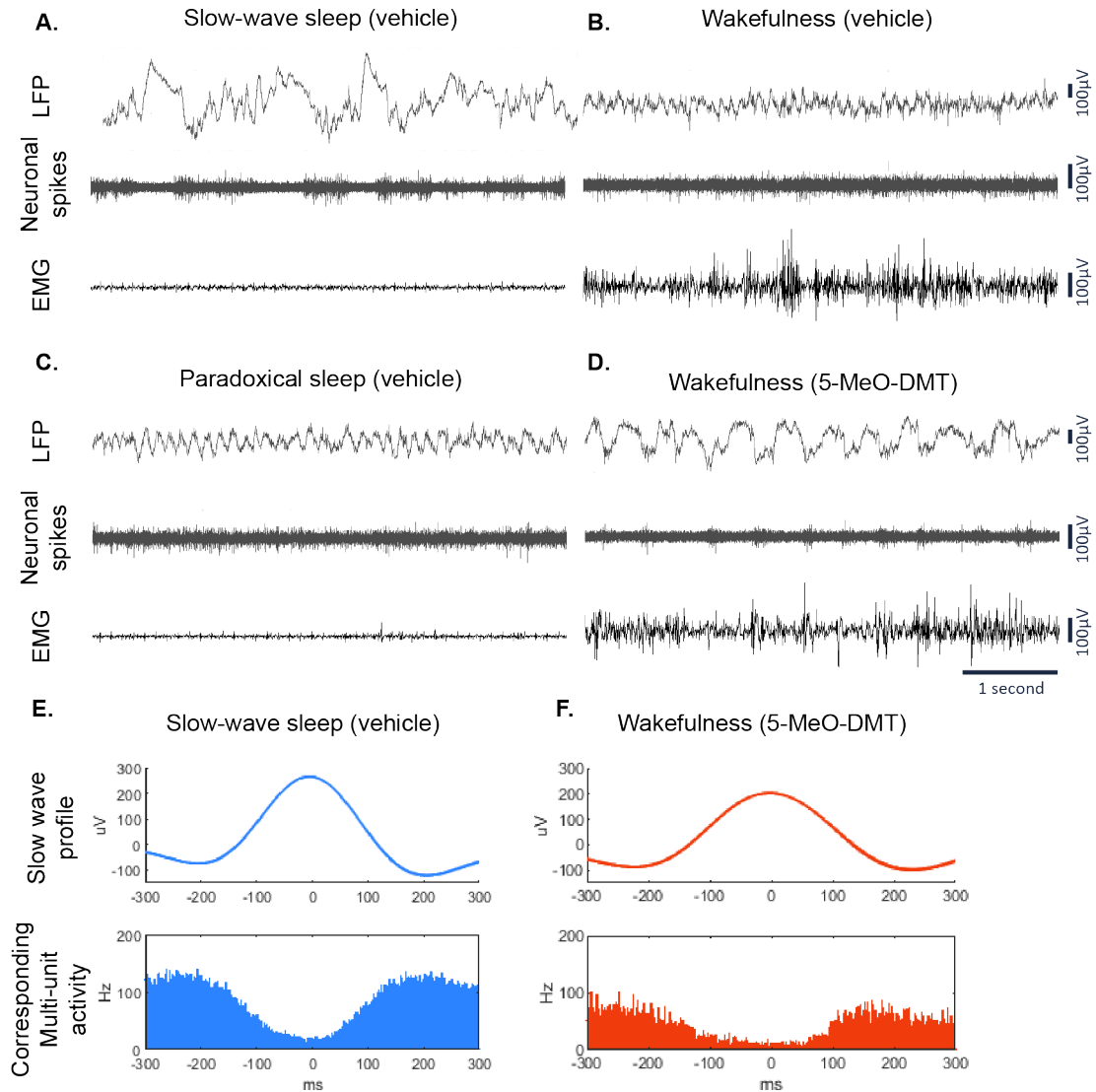
EEG theta activity (7 Hz to 12.5 Hz) (ME analysis, frequency\*condition:  $F_{152,908} = 14.15$ ,  $p < 0.0001$ , Fisher's post hoc  $p < 0.05$ ), resulting in a SWS-like pattern of EEG activity (Figure 3.6.B). The activity patterns were indistinguishable between drug and vehicle 1 hour following the injections in the frontal derivation (ME analysis, frequency\*condition:  $F_{154,1074} = 3.29$ ,  $p < 0.0001$ ; Fisher's LSD  $p > 0.05$  for the frequencies of interest) (Figure 3.6.C), and in the occipital derivation where a marginal difference was found only between 3 – 3.5 Hz (ME analysis, frequency\*condition:  $F_{152,908} = 3.41$ ,  $p < 0.0001$ ; Fisher's LSD  $p < 0.05$  between 5-MeO-DMT and vehicle 3Hz to 3.5 Hz) (Figure 3.6.D).

We then isolated the frontal and occipital EEG delta activity and the occipital EEG theta activity to calculate their time courses. The EEG frontal and occipital delta activity was distinctly increased in the first 30 minutes following the drug injection (Figure 3.6.E-F), with the occipital EEG theta activity heavily suppressed during the same time window (Figure 3.6.G).

The possibility remains that the slowing down of the EEG global activity after an injection of 5-MeO-DMT merely reflects abnormal or artefactual brain signals, unrelated to physiological slow-wave activity, typical for sleep. To address this possibility, we carefully inspected the LFP and corresponding multi-unit activity (MUA), which were recorded in a separate subset of animals in V1. It is well-known that slow waves during physiological sleep are accompanied by the occurrence of OFF-periods – which are generalised periods of synchronised neuronal silence, when the recorded population of neurons do not emit action potentials, typically lasting 100-200 ms (Harding, 2022; Kahn et al., 2022).

As expected, after vehicle injection, LFPs and MUA showed key signatures of wakefulness and SWS, whereby the latter was characterised by frequent OFF-periods during LFP slow waves (Figure 3.7.A). In contrast, OFF-periods were rare during waking or PS, following an injection of vehicle (Figure 3.7.B-C). 5-MeO-DMT injection resulted in the occurrence of prominent LFP slow waves during





**Figure 3.7: Intracortical activity in the visual cortex after an injection of 5-MeO-DMT.** **A.** Representative LFP and corresponding MUA in the primary visual cortex and nuchal EMG of one animal during SWS following a vehicle injection over 5 seconds. **B.** Representative LFP and corresponding multi-unit activity (MUA) in the primary visual cortex and nuchal EMG of the same animal 5 minutes after a vehicle injection over 5 seconds. **C.** Representative LFP and corresponding MUA in the primary visual cortex and nuchal EMG of the same animal during PS following a 5-MeO-DMT injection over 5 seconds. **D.** Representative LFP and corresponding MUA in the primary visual cortex and nuchal EMG of the same animal 5 minutes after a 5-MeO-DMT injection over 5 seconds. **E.** Average slow wave and SEM (top) and corresponding MUAs (bottom) for automatically detected slow waves occurring during any 30 minutes of SWS happening after a vehicle injection. **F.** Average slow wave and SEM (top) and corresponding MUAs (bottom) for automatically detected slow waves happening during all episodes of wake within 30 minutes after an injection of 5-MeO-DMT.



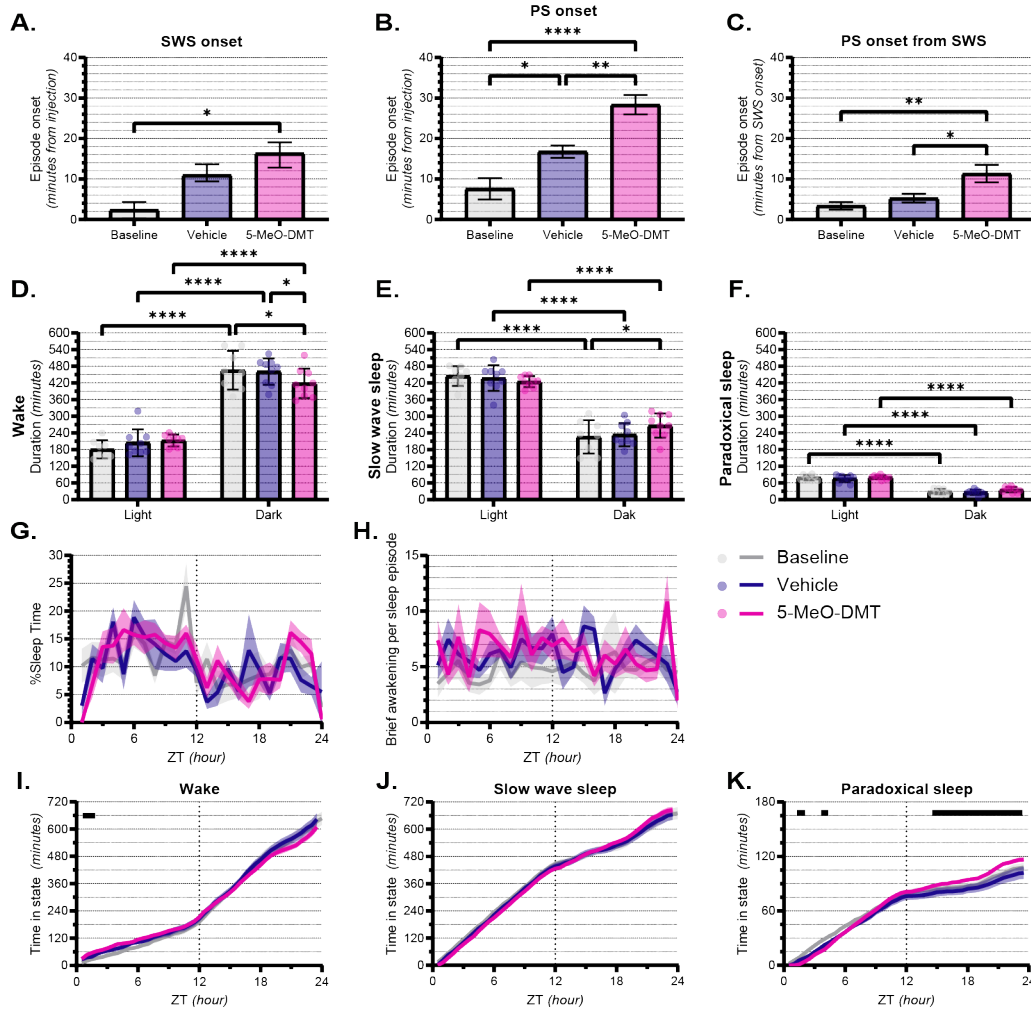
wakefulness, resembling those occurring in SWS, and were invariably accompanied by neuronal OFF-periods (Figure 3.7.D).

To further characterise the relationship between LFPs and neuronal activity during sleep and after 5-MeO DMT, we detected individual LFP slow waves in both conditions and calculated the corresponding average MUA. LFP slow waves were calculated from the best LFP channels out of the 16 available for all animals, along with the corresponding MUA. We observed that in both SWS (Figure 3.7.E) and wake after 5-MeO-DMT (Figure 3.7.F), the average LFP slow wave was accompanied by a strong suppression of MUA, characteristic of sleep-like OFF-periods.

### 3.3.3 5-MeO-DMT alters sleep architecture but not sleep signals

With a clear effect on wake behaviour and brain signals, we then focussed on the effect of 5-MeO-DMT on the episodes of sleep following the drug injection. We first analysed the architecture of sleep, and then EEG and LFP activity. We first found that SWS was significantly delayed following an injection of 5-MeO-DMT compared to baseline (Friedman test  $\chi^2(2) = 7.75$ ,  $p < 0.05$ ; Dunn's post-hoc  $p < 0.05$ ) but not compared to vehicle (Dunn's post-hoc  $p = 0.95$ ), as the first episode lasting longer than 1 minute appears on average  $17.02 \pm 2.07$  minutes after the injection of 5-MeO-DMT, as opposed to  $11.52 \pm 3.03$  minutes after the injection of saline, and  $4.20 \pm 2.33$  minutes after light onset in the case of baseline (Figure 3.8.A).

PS, however, was significantly delayed after an injection of 5-MeO-DMT compared to baseline (RM-one-way ANOVA  $F_{2,14} = 25.22$ ;  $p < 0.0001$ , Tukey's post hoc  $p < 0.0001$ ) and vehicle ( $p < 0.01$ ), PS was also significantly increased after an injection of vehicle compared to baseline ( $p < 0.05$ ), but not as much as 5-MeO-DMT (Figure 3.8.B). Then, PS onset in relation to SWS onset was calculated, and we found a significant delay in PS onset in the 5-MeO-DMT condition compared to both baseline and vehicle (RM-one-way ANOVA,  $F_{2,14} = 7.16$ ;  $p < 0.01$ , Tukey's post hoc  $p < 0.05$  for 5-MeO-DMT against baseline or vehicle). These results

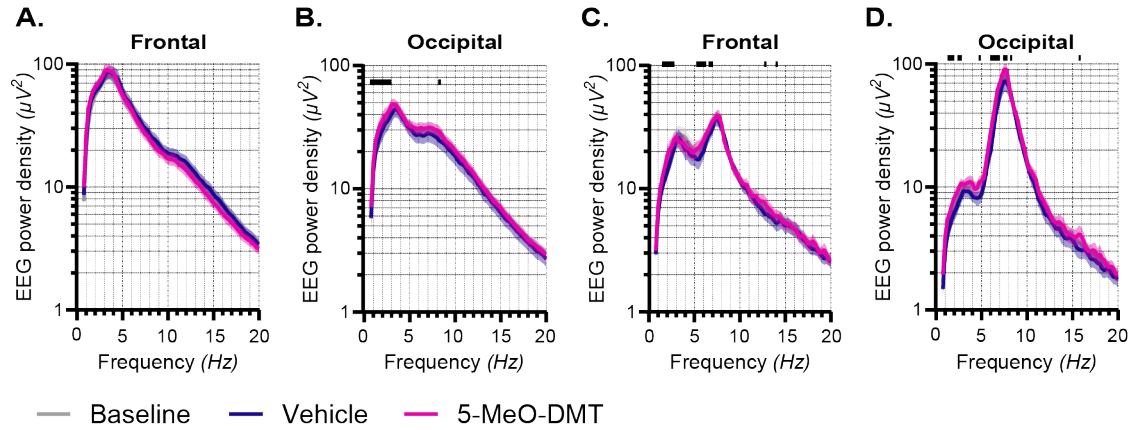


**Figure 3.8: Changes in sleep architecture following 5-MeO-DMT administration.** A-B. Time from the injection to the first occurrence of a consolidated SWS (< 1 minute; A.) and PS (< 32 s; B.) episode after an injection of 5-MeO-DMT or vehicle at light onset, compared to baseline (light onset only). C. Time from SWS onset to PS onset after an injection of 5-MeO-DMT or vehicle at light onset, compared to baseline. D-F. Average episode duration for wake (D.), SWS (E.) and PS (F.) in the light period or the dark period between all conditions. G. Time course of the percent of sleep time spent in PS over 24 hours. H. Time course of the amount of brief awakening per sleep for 24 hours. Injections time is ZT = 0. I-K. time course of cumulative time in wake (I), SWS (J) or PS (K) for all conditions. All data are mean + SEM except for A. median + IQRs. (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\*\* $p < 0.0001$ , a line denotes a  $p < 0.05$  at the given time points). N = 12 for all panels. See Table A.5, Table A.6, & Table A.7 for statistical analyses.

suggest that the animals treated with 5-MeO-DMT took longer to fall asleep, and even longer to enter a consolidated episode of PS, reaching it  $11.33 \pm 2.146$  minutes after SWS onset after an injection of 5-MeO-DMT, compared to  $3.38 \pm 0.91$  minutes and  $5.25 \pm 1.08$  minutes for baseline and vehicle conditions respectively (Figure 3.8.C).

Subsequently, the amount of time spent in waking, SWS and PS were analysed. As expected, the light and dark period had an effect on the time spent in each state. ME analysis showed an effect of the light period on wake, with the animals spending significantly more time in wake in the dark period than in the light period ( $F_{1,9} = 210.4$ ,  $p < 0.0001$ ) (Figure 3.8.D). The opposite was true for SWS and PS, the animal spending more time in SWS and PS during the light period (SWS:  $F_{1,9} = 159.1$ ,  $p < 0.0001$ ; PS:  $F_{1,9} = 193.9$ ,  $p < 0.0001$ ), regardless of the condition (Tuckey's post hoc, light period versus dark period in baseline, vehicle and 5-MeO-DMT  $p < 0.0001$ ) (Figure 3.8.E-F). However, an effect of interaction between the light period and condition was found, with significantly less wake during the dark period in the 5-MeO-DMT condition (light\*condition:  $F_{2,10} = 13.21$ ,  $p < 0.01$ ; Tuckey's post hoc  $p < 0.05$  for 5-MeO-DMT against baseline and vehicle), leading to significantly more SWS in the dark period in the 5-MeO-DMT condition (light\*condition:  $F_{2,10} = 8.82$ ,  $p < 0.01$ ; Tuckey's post hoc  $p < 0.05$  for 5-MeO-DMT against baseline and vehicle) (Figure 3.8.D-E).

Sleep overall architecture was also analysed in terms of proportion of time asleep spent in PS, and occurrence of brief awakening per sleep episode. ME analysis showed an effect of interaction between ZT time and condition, showing marginal differences between baseline and both injection conditions suggesting a potential effect of the injection at 1 hour and at 11 hour post injection (ME analysis, ZTtime\*condition  $F_{46,211} = 1.45$ ,  $p < 0.05$ ; Tuckey's post hoc  $p < 0.05$  for baseline against 5-MeO-DMT or vehicle), but no difference was found between the vehicle and the 5-MeO-DMT condition (Figure 3.8.G). Previous studies suggested that sleep



**Figure 3.9: Changes in sleep EEG activity following 5-MeO-DMT administration.** **A-B.** Mean EEG power of 2 hours, from 1 hour after a 5-MeO-DMT injection or a vehicle injection or averaged over 24 hours (baseline) of SWS epochs in the frontal (A.) and occipital (B.) derivations. **C-D.** Mean EEG power of 2 hours, from 1 hour after a 5-MeO-DMT injection or a vehicle injection or averaged over 24 hours (baseline) of PS epochs in the frontal (C.) and occipital (D.) derivations. (Black horizontal line denotes  $p < 0.05$  at the given frequency).  $N = 12$  for all panels. See Table A.8 for statistical analyses.

was more fragmented after an injection of a psychedelic (Thomas et al., 2022), so we therefore calculated sleep fragmentation as the number of brief awakening epochs per episode of sleep and found a significant difference between the conditions (ME analysis effect of condition:  $F_{2,18} = 6.79$ ,  $p < 0.01$ ) but not an interaction between ZT time and condition (ZTtime\*condition  $F_{46,211} = 0.93$ ,  $p = 0.60$ ) (Figure 3.8.H).

To further investigate the dynamics of states we also measured the cumulative time spent in each state. The animals spent more time in wake in the first hour in the 5-MeO-DMT condition compared to the vehicle condition (ME analysis, ZTtime\*condition  $F_{92,644} = 3.70$ ,  $p < 0.05$  (GG), Tukey's post hoc  $p < 0.05$  Zt = 1 in 5-MeO-DMT vs vehicle) (Figure 3.8.I). No difference was found between all conditions in SWS (Figure 3.8.J). For PS however, an injection of 5-MeO-DMT led to less PS than the vehicle in the first 2 hours and between 3 and 4 hours, but significantly more time was spent in PS from 16 hours onwards (ME analysis, ZTtime\*condition  $F_{46,211} = 1.45$ ,  $p < 0.05$ ; Tukey's post hoc  $p < 0.05$ ) (Figure 3.8.K).

We finally looked at EEG and LFP brain activity during both SWS and PS to

measure a potential acute effect of 5-MeO-DMT. Because the first hour of recordings following the injection of either 5-MeO-DMT or vehicle is strongly dominated by long episodes of waking, we selected only consolidated episodes of SWS and PS that occurred 1 hour after the injection and up to 3 hours after to focus on the stable episodes of SWS and PS as close to the injection as possible. Significant, albeit small, increases of the occipital delta frequencies were noted during SWS (ME analysis, frequency\*condition:  $F_{154,920} = 4.38$ ,  $p < 0.0001$ ; Fisher's post hoc  $p < 0.05$ ), and in the delta and theta frequencies of the frontal (ME analysis, frequency\*condition:  $F_{154,1074} = 1.93$ ,  $p < 0.0001$ ; Fisher's post hoc  $p < 0.05$ ) and occipital derivation during PS (ME analysis, frequency\*condition:  $F_{154,920} = 1.34$ ,  $p < 0.01$ ; Fisher's post hoc  $p < 0.05$ ), broadly suggesting the same EEG activity patterns in the subsequent episodes of sleep (Figure 3.9).

## 3.4 Discussion

### 3.4.1 The changes in waking behaviour

To first assess whether 5-MeO-DMT had an effect in laboratory mice, we measured HTR as a well-known behaviour occurring after administration of psychedelics and whose occurrence is often correlated with the potency of psychedelics in humans (Halberstadt et al., 2020). As opposed to human studies capable of assessing the experience directly with a questionnaire, HTR is one of the most reliable read-outs of 5-HT<sub>2A</sub> activation in mice and thus provide a good estimate to a psychedelic state in laboratory mice. Our results are in line with these notions. 5-MeO-DMT transiently increased the amount of HTR following a time course with a peak at 5 minutes post-injection and 15-20 minutes half-life observed in mice (Shen et al., 2009).

The HTR following an injection of serotonergic psychedelic is mediated through the 5-HT<sub>2A</sub> receptor (González-Maeso et al., 2007), like the potency in human of *classical* psychedelics (Vollenweider et al., 1998). A surprising result was obtained using the non-hallucinogenic 5-HT<sub>2A</sub> agonist Tabernanthalog (TBG), analogous to ibogaine from *Tabernanthe iboga*, suggesting a stronger correlation between the

human psychedelic experience and HTR in rodents. Indeed, TBG is not known to induce any psychedelic experience in humans despite being a 5-HT<sub>2A</sub> receptor agonist, and it also does not trigger any HTR in rodents (Cameron et al., 2021). In the study by Cameron and colleagues, the authors also used a 10 mg/kg dose of 5-MeO-DMT as a positive control which elicited between 15 to 30 HTR over the recorded period, which is in line with our data using a lower dose. It also supports the notion that a higher dose of a psychedelic elicits a stronger HTR (Cameron et al., 2021). However, the HTR assay currently necessitates manual scoring of video recordings which might lead to a strong experimenter bias as described later. Automated scoring methods using accelerometers are currently being developed to improve the detection of HTR and may provide more reliable results.

Other than the HTR, the behaviour of the animals after injection of 5-MeO-DMT was also altered in other ways. While the drug-treated animals were undoubtedly awake and behaviourally active, the goal of their spontaneous behaviour was changed in a noticeable way. In subsection 3.3.1, it was mentioned that their exploratory behaviour targeted the immediate surroundings of the animal. No abnormalities were noted in locomotion, as supported by the observations in the running-wheels.

Our results therefore suggest that the dose of 5-MeO-DMT used in this chapter (and in the subsequent chapters) is high enough to induce changes, but low enough so that these changes in behaviour observed would not be associated with the SS, which can be the case with other psychedelics (Sun-Edelstein et al., 2008). In rodents, the SS consists mostly of hind limb abduction and head weaving, but also, in rare cases, backward walking, straub tail, tremors, low body posture, hyperactivity and decreases in body temperature (Haberzettel et al., 2013). We did observe instances of backward walking, but by and large, the behaviour of the animals was normal. Specifically, our experiments with running wheels did not reveal abnormalities in locomotor activity, and we never observed hind limb abduction or flat body posture.

The changes in behaviour, however, were highly stereotypical across the drug-treated animals and while attempts were made to keep the experimenter blind to the

drug/vehicle condition of the mice, this could not be achieved with visual scoring. Indeed, within 5 minutes of monitoring the videos, the scorer could accurately and reliably determine which animals had received which treatment. Hence, we believe that the current visual assessment of the effects of psychedelics on behaviour is subject to strong bias and other scoring methods should be investigated. The use of a video-recording set-up of higher quality could yield valuable data in this regard and might allow automatic scoring using machine-learning algorithms. While HTR do indeed provide a good read-out of psychedelic potency, it remains a macroscopic behaviour. To the best of our knowledge, behaviours at a lower scale such as twitches of the limbs or middle ear muscle twitches such as the ones measured during PS (Pessah & Roffwarg, 1972) have not been investigated.

### **3.4.2 The changes in goal-oriented behaviour**

As mentioned earlier, the target of exploratory behaviour seems altered by the 5-MeO-DMT injections. Indeed, spontaneous exploratory behaviours after the administration of 5-MeO-DMT are oriented towards the immediate surroundings of the animals, and the exploration induced by the positioning of a bowl of sugar pellets in the environment is strongly affected. The results presented in this chapter could suggest a potential anhedonic property of 5-MeO-DMT with the animals failing to engage with the sugar pellets for a significant amount of time. As 5-HT is known to modulate the regulation of numerous behaviours including appetite (Müller & Cunningham, 2020), it is maybe not surprising to observe such an effect. The delay of exploring the bowl could therefore represent a lack of interest in its content. However, such changes could also be the result of ongoing alterations to sensory processes.

The results of drug-discrimination studies however suggests that appetite for food reward is not completely gone after an injection of 5-MeO-DMT, and that laboratory animals can perform cognitively demanding tasks after administration of psychedelics for appetitive rewards (Ermakova et al., 2022; Hanks & González-Maeso, 2013). The drug discrimination tasks suggest that the internal perception of

wakefulness is indeed altered, as the animals correctly identify when they have been injected with 5-MeO-DMT. It also suggests that this alteration can be bypassed by cognitively demanding tasks.

It is thought that the human psychedelic experience is environment dependent. Theorised by Al Hubbard and democratised by Leary (Pollan, 2019), the idea that the mindset and expectations of the participant as well as the setting and general environment of the studies providing context-dependant alteration is argued to play a leading role in the therapeutic properties of psychedelics (Carhart-Harris et al., 2018b). Our result suggests that, while the behaviour of the animal was undoubtedly wake-like, there might be strong alterations in perception and interactions with the environment. With the increased latency to pellet-eating behaviour, we would expect to observe a lack of participation or major delay in reward-collecting behaviour. As this is not the case, the possibility remains that the occurrence of the task itself might represent a strong change in context capable of downsizing the effects of psychedelics. Therefore, psychedelics could induce an altered internal state different from normal wakefulness which can be at least modulated by the presence of external stimuli.

### 3.4.3 A paradoxical wake state

The highly unusual features of the state induced by the psychedelic 5-MeO DMT in mice led us to call it “paradoxical wakefulness” (PW), as it was a hybrid state, precisely the opposite to the characteristics of PS. Specifically, while in PS high brain activity dominated by the theta-rhythm is accompanied with low EMG tone, PW was instead associated with SWS-like cortical activity in actively moving mice, with elevated EMG activity. Furthermore, theta-frequency activity was heavily suppressed in PW, while combined LFP and MUA recordings during PW revealed an occurrence of OFF-periods typical for slow waves during SWS.



While the relationship between SWS, PS and PW remains to be clarified, we speculate that the occurrence of PW may contribute to the delay in PS typically observed after administration of psychedelics such as LSD, psilocybin, and psilocin (Dudysová et al., 2020; Thomas et al., 2022). One possibility is that PW alters the homeostatic mechanism responsible for the regulation of the sleep/wake cycle, diminishing the propensity for PS (Benington & Heller, 1994; Franken, 2002). While it is well recognised that wakefulness is a highly dynamic and heterogenous state, often having subtle features of SWS, for example during quiet states or after sleep deprivation, the state we refer to as PW is an unusually extreme case of state dissociation, akin to parasomnias (Castelnovo et al., 2018). The occurrence of slow-wave activity during normal waking behaviour has been reported before, as well as periodic alternation of distinct sub-states of wakefulness in conjunction with spontaneous locomotor activity in mice (Vanderwolf & Robinson, 1981; Vyazovskiy & Tobler, 2012). Our current observations further highlight an under-appreciated capacity to manifest hybrid states, such as PW, although whether global dissociation like PW can occur spontaneously in normal animals and under what conditions, remains to be determined. At any rate, the existence of such a paradoxical brain state during wake supports the notion that SWA, thought of as a key defining feature and a hallmark of SWS in human and rodent sleep research, is not a feature exclusive to sleep, and its causal link with states of consciousness, sleep depth or sleep functions must be reconsidered (C. J. Davis et al., 2011; Funk et al., 2016; A. M. Stephan et al., 2021).

#### **3.4.4 5-MeO-DMT and sleep**

The delay in SWS onset and suppression of PS measured here supports previously published observations (Barbanj et al., 2008; Dudysová et al., 2020; Thomas et al., 2022; Wichniak et al., 2017). However, we did not observe significant changes in SWS stability, contrary to other studies addressing the effects of psychedelics (Thomas et al., 2022). We hypothesise that this could be due to the short-lasting

effects of 5-MeO-DMT compared to other psychedelic drugs. It is important to note that the initial wakefulness observed might not be directly caused by 5-MeO-DMT, as an injection of vehicle was also followed by an increase in the quantity of wake behaviour and might therefore be caused by injection stress. Whether this observed stress is elevated further by the psychedelic properties of 5-MeO-DMT remains to be analysed. Psychedelics have been shown to have wake-promoting properties (Thomas et al., 2022), and therefore psychedelics with a longer half-life like psilocin might still promote some psychedelic-wake state and prevent the occurrence of consolidated SWS. Therefore, a short-lasting psychedelic like 5-MeO-DMT could confine the psychedelic state to an initial wake-state, allowing the following SWS episodes to be more similar to physiological sleep.

While PS is delayed by the injection of 5-MeO-DMT, the rebound shown several hours later is likely due to PS homeostasis (Benington & Heller, 1994; Franken, 2002). We found a significant difference in the cumulative time in PS at 20 to 24 hours after the injection of 5-MeO-DMT. As psychedelics are reported to have enduring effects on brain plasticity and mood effects, it would be interesting to analyse whether these are accompanied by changes in the sleep/wake cycle in the days or weeks following the injection, and whether longer-acting psychedelics, such as LSD and short-acting compounds, such as 5-MeO-DMT, differ in this respect. Furthermore, it is important to optimise the route of delivery of the drug, as an acute intraperitoneal injection may be stressful and alter both the immediate and delayed effects of these compounds.

### 3.4.5 Further analyses

The regulation of 5-MeO-DMT-induced slow-waves remains to be investigated, specifically its sensitivity to sleep pressure, given the well-recognised notion that SWA during SWS reflects preceding sleep-wake history (Borbély, 1982; Vyazovskiy et al., 2009a). Likewise, it remains unknown whether the network oscillatory activity during PW arises from the same cellular and network mechanism as the

slow oscillation during sleep (C. J. Davis et al., 2011; Vanderwolf & Robinson, 1981; Vyazovskiy et al., 2009b).

We observed a profound suppression of theta activity in the occipital derivation during active wakefulness, when it is typically present in exploring animals. Theta activity is mediated by the medial septal nucleus, projecting towards the hippocampus where rhythmical activity has been linked to waking behaviour, notably spatial and temporal processing (Buzsáki et al., 1981; O’Keefe, 1993; O’Keefe & Recce, 1993; Vanderwolf & Robinson, 1981; Zhan, 2015). Intriguingly, human psychedelic experience is associated with altered processing of time and space (Barsuglia et al., 2018). We therefore hypothesise that suppression of theta activity during PW would be associated with a disruption of the processing of spatial and temporal information.

Studies in rodents suggest psychedelic drugs such as LSD alter the firing rates of hippocampal neurons, strongly implicated in the expression of the theta rhythm (Domenico et al., 2021). Taken together, the alteration of the EEG activity pattern observed here after injection of 5-MeO-DMT could be due to reorganisation within neuronal networks. Cortical activity patterns are tuned by changing concentrations of a wide range of neuromodulators, such as acetylcholine, 5-HT, and norepinephrine (McGinley et al., 2015). Whether the state described here is accompanied by altered levels of other wake- or arousal-promoting neuromodulators remains to be determined.

The altered sleep architecture also deserves more attention. Indeed, while the electrophysiological experiments presented in this chapter were conducted with injections at light onset to maximise the chances of monitoring a sleep episode immediately following the state induced by 5-MeO-DMT, injections at dark onset would be more likely associated with an active behaviour and low sleep pressure. Such injections would theoretically be at a time point which is the furthest possible to PS onset. The question of PS expression in these conditions remains totally

open.

### 3.4.6 Conclusion

The results presented in this chapter report the acute effects of 5-MeO-DMT injection on vigilance states, spontaneous behaviour, and brain activity in freely moving mice. 5-MeO-DMT induced a prominent change in EEG and LFP during wakefulness, as reflected in the occurrence of sleep-like slow waves associated with neuronal OFF-periods, while theta-frequency activity was markedly suppressed. Despite sleep-like patterns of brain activity, the behaviour of the animals was typical for wakefulness, as reflected in the occurrence of grooming, exploring, and running, and no abnormalities in body posture were noted. We also observed that exploration, when it was present, was mostly targeted at the immediate surroundings of the animal. The effects were short-lasting, and dissipated within an hour, consistent with the pharmacokinetics of 5-MeO-DMT. Here, we report a state that supports the notion that the serotonergic system can modulate the quality of states rather than, or in addition to, their quantity. From this chapter, three points remain to be explored. First, the levels of attention and arousal, second, the cortical response to external sensory stimuli, and finally the regulation of psychedelic-induced slow-waves. These elements will be discussed in the following chapters.

# 4

## The development of new methods to measure attention and arousal

### Contents

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|            |                                                                    |            |
|------------|--------------------------------------------------------------------|------------|
| <b>4.1</b> | <b>Introduction</b>                                                | <b>92</b>  |
| 4.1.1      | The monitoring of attention in rodents                             | 92         |
| 4.1.2      | Measuring arousal with pupillometry                                | 95         |
| 4.1.3      | Experimental aims                                                  | 98         |
| <b>4.2</b> | <b>Methods</b>                                                     | <b>99</b>  |
| 4.2.1      | Animal husbandry                                                   | 99         |
| 4.2.2      | Food restriction                                                   | 101        |
| 4.2.3      | Behavioural paradigm                                               | 101        |
| 4.2.4      | Sleep deprivation                                                  | 105        |
| 4.2.5      | Oculometry                                                         | 105        |
| 4.2.6      | Injections                                                         | 107        |
| 4.2.7      | Analysis                                                           | 107        |
| <b>4.3</b> | <b>Results</b>                                                     | <b>108</b> |
| 4.3.1      | Sleep deprivation does not alter attention                         | 108        |
| 4.3.2      | The development of the oculometre                                  | 111        |
| 4.3.3      | 5-MeO-DMT injections induces pupil mydriasis in freely moving mice | 113        |
| <b>4.4</b> | <b>Discussion</b>                                                  | <b>118</b> |
| 4.4.1      | The monitoring of attention in mice                                | 118        |
| 4.4.2      | A novel approach to oculometry and behaviour monitoring            | 121        |
| 4.4.3      | The implications of pupil mydriasis                                | 123        |
| 4.4.4      | Conclusion                                                         | 125        |

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## 4.1 Introduction

### 4.1.1 The monitoring of attention in rodents

Attention can be defined as “The action, fact, or state of giving heed; earnest direction of the mind, consideration” (“Oxford English dictionary”, 2024) and is closely related to vigilance. In humans, being vigilant is linked to sustaining attention for an undefined length of time (Oken et al., 2006). The measure of attention in animal models corresponds to measuring a behaviour in relation to a given stimulus.

In both humans and animals, electrophysiological investigations on attention were conducted in the 1960s. In humans, it is possible to divert the attention of the subject by asking the subject to change its focus (M. Jouvett et al., 1959a). Early studies in cats claimed to monitor the electrophysiological response to attention change by presenting a distraction from the auditory clicks to the animals (Hernandez-Peon et al., 1956), however due to the lack of reproducibility, it was later demonstrated that these changes were due to changes in the position of the head, leading to changes in the sound amplitude reaching the eardrum (Gazzaniga et al., 2009). Later studies showed that attention is regulated by both endogenous and exogenous mechanisms (Pashler et al., 2001). The endogenous, top-down component suggesting a cognition-driven mechanism would play out jointly with an exogenous stimulus-driven mechanism. Pashler (1990) conducted a review of the 1990s literature and suggested an ecological basis to these mechanisms of attention, focussing on the involuntary component of attention shifts (Pashler, 1990).

Attention has been extensively studied in humans and animals (Robbins, 2002; van Enkhuizen et al., 2014). To measure attention in clinical settings, Rosvold and Mirsky developed two continuous performance tests where, for 10 minutes, the subjects were asked to press a button every time the letter X appeared in the first task, or every time the letter X was following the letter A (Beck et al., 1956; Mirsky & Rosvold, 1960).

Another tool to measure attention was derived from this task in 1959 by Leonard and was called the 5-Choice Serial Reaction Time (5-CSRT) task (Leonard, 1959; Mar et al., 2013; Robbins, 2002). In this task, the participant is asked to report the location of a visual target amongst five others, and, upon selection, another location would be picked at random for the target to be. The appeal of this technique resides in the measurement of rate of behavioural outputs, but also the efficiency (Robbins, 2002).

As described in rats for an equivalent 9-CSRT task, a light was projected briefly onto holes. The rat had to nose-poke the correct hole once the stimuli was off before collecting a reward. The task also scored omission when the rat failed to report during a given time, and error when the rat poked the wrong hole (Carli et al., 1983). Thus, rate of accuracy (correct over incorrect responses) and the rate of omission (omissions over total responses) were measured. This particular setup developed by Carli required a trial initiation nose poke in the reward chamber allowing the existence of premature responses, dropping the chance performance rate to 20% (Carli et al., 1983; Robbins, 2002). Because of this initiation, the muscle activity to initiate a trial and the one required for a correct performance was the same, thus a motor defect is not sufficient to explain an impairment in the rate of accuracy and therefore a drop in accuracy reflects impaired attention. The 5-CSRT task can also measure modalities. Motivation can be read through the latency to collect the reward, and compulsive behaviours with the occurrence of premature responses (Evenden, 1999; Robbins, 2002). Robbins, however reminds that response latency for correct responses can be influenced by motivational, sedative or motor factors (Robbins, 2002).

Sleep deprivation is a process known to alter attention. Indeed, in experiments using similar paradigms such as the psychomotor vigilance task, it has been shown that sleep deprivation decreases attention in humans as sleep deprivation increased lapses and a night of recovery sleep restored baseline level of performances (Dinges et al., 1997). A study conducted on both animals and rodents and the 5-CSRT

task showed that sleep deprivation impaired false target and hit rate in humans, and increased the omission rate in mice, thus suggesting an overall impairment of attention (van Enkhuizen et al., 2014). However, the author notes that there is an overall decrease of amount of trials after sleep deprivation, and that the impairments induced by sleep deprivation were only significant in good performers, suggesting that beside attentional deficit, sleep deprivation might also alter motivation.

The serotonergic system is thought to be involved in the modulation of attentional processes (Robbins, 1997; Soubri , 1986). Indeed, increased serotonin levels following SSRI administration have been previously correlated with deficits in sustained attention (O’Hanlon et al., 1998). In humans, experiments in fMRI also successfully linked increased serotonin level and impaired sustained attention by decreasing the activity of prefrontal brain areas suggesting that imbalance in serotonin levels in both directions negatively impacts performance in attention-related tasks (Wingen et al., 2008). In the 5-CSRT task however, manipulation of the 5-HT system showed mixed results (Robbins, 2002).

In rats, global depletion of 5-HT did not alter the rate of correct responses and omissions but did increase the amount of premature responses and the speed to retrieve the reward, suggesting that global 5-HT levels were more involved in the regulation of impulsive behaviours rather than attentional processes (Harrison et al., 1997a). However, lesions in the median and dorsal raphe nuclei showed different results. Lesions in the median raphe nucleus showed responses comparable with global 5-HT depletion, while lesions in the dorsal raphe nucleus increased accuracy and the speed to induce a correct response, and decreased the omission rate (Harrison et al., 1997b).

On the opposite side of the spectrum, elevated 5-HT activity in the prefrontal cortex have been shown to decrease the rate of accuracy while also, paradoxically, increase the number of premature responses (Puumala & Sirvi , 1998). It was also suggested that 5-HT manipulation might interact with other neurotransmitters such as dopamine and acetylcholine which can in turn enhance attentional performances



(Robbins, 2002).

In rodents, the 5-CSRT task has shown that psychedelics induce a deficit in sustained attention through activation of 5-HT<sub>2</sub> and 5-HT<sub>1</sub> receptor (Carli & Samanin, 1992; Koskinen et al., 2000a, 2000b; Robbins, 2002). In rats, the administration of LSD (5-HT<sub>2A</sub> receptor agonist at 0.1 mg/kg), quipazine (5-HT<sub>2</sub> receptor agonist at 2.5 mg/kg) and d-fenfluramine (5-HT<sub>1</sub> receptor agonist at 1.25 mg/kg) all significantly increased the omission rate, while LSD and quipazine decreased the percent of correct responses (Carli & Samanin, 1992). It was found that the increased rate of premature responses induced by DOI administration at various concentration was reverted by the 5-HT<sub>2A</sub> receptor antagonist ketanserin (Koskinen et al., 2000a, 2000b).

However, such studies report important limitations. For example, after administration of the drugs, lower overall performance has been observed, which the author explain by a reduced motivation in the food-rewarded task due to previously-reported hypophagia or the occurrence of repetitive, autoshaped behaviour (Carli & Samanin, 1992; Koskinen et al., 2000a, 2000b).

To date, no study on the effects of 5-MeO-DMT on attention has been performed. As most of the previous study have been focussed on the 5-HT<sub>2A</sub> receptor agonists and showed that this receptor mostly influences impulsive behaviour, the administration of a 5-HT<sub>1A</sub> agonist might have effects similar to a global reduction of 5-HT levels. However, due to potential limitation, this part of the project was not realised and instead the focus was set on measurement of arousal.

#### **4.1.2 Measuring arousal with pupillometry**

Arousal differs from attention as it generally refers to a state transition towards wakefulness, although arousal levels can change dramatically across the wake state. It is maintained by the ascending reticular activating system originating in the brainstem as well as the lateral hypothalamus and the tuberomammillary nucleus, as presented in Chapter 1, and relies on monoaminergic projections amongst other,

including but not limited to dopaminergic neurotransmission from the ventral tegmental area (VTA), noradrenergic (NA) neurotransmission from the LC, 5-HT neurotransmission from the DRN (Eban-Rothschild et al., 2016; Robbins, 1997).

Indeed, it has been shown that neuronal activity from these areas heavily correlates with changes in vigilance state, with 5-HT release being high during wakefulness and decreasing with quiescence (Carlson, 2016); LC firing and NA release have been correlated with the infra-slow oscillatory fluctuations of arousal levels (Carro-Domínguez et al., 2023; Osorio-Forero et al., 2021); and the VTA firing and dopamine activity in the nucleus acumens have been correlated with the maintenance of wake-like cortical activity (Eban-Rothschild et al., 2016). As levels of arousal heavily fluctuate during wake and sleep, the use of external cues to increase arousal from sleep to wake can be used as a measure of objective sleep depth, with the intensity of said cue marking the arousal threshold (Bonnet et al., 1978; Robbins, 1997).

The arousal levels can change during wake state. It is possible to monitor such changes by measuring levels of neurotransmitters (Carlson, 2016). However, these variation in levels of neurotransmitters can be associated with changes in the autonomic nervous system and the endocrine system (Bradley et al., 2008). A common autonomic change is the variation of the pupil size through the activation of NA- $\alpha 2$  receptors hence why, in mammals, an increase in pupil diameter (mydriasis) is associated with elevated NA levels and elevated arousal levels. (Bradley et al., 2008; Ebitz et al., 2014). Recent studies measuring pupil size throughout sleep and wake showed that pupil diameter can be a predictable marker of vigilance states and NA levels in both rodents and humans, with a dilation of the pupil occurring during wake states and a constriction during sleep, with the smallest pupil size during paradoxical sleep (Carro-Domínguez et al., 2023; Yüzgeç et al., 2018).

Psychedelic use in human leads to self-report of increased arousal with very few studies measuring variation of pupil size. The alteration of arousal by psychedelics often peaks with the sensation of ego-dissolution, the loss of perception of one's self in

an increased sense of boundlessness (Mason et al., 2020; Preller & Vollenweider, 2018) together with the occurrence of an ecstatic state (Fischer et al., 1970). Surprisingly, these changes in arousal might not be linked to the 5-HT<sub>2A</sub> receptor (Carter et al., 2007), suggesting a possible 5-HT<sub>1A</sub>-mediated alteration of arousal by psychedelics. Notably, only anecdotal case reports mention analysis of pupil diameter and vision in psychedelic users or in animals, and no complete pupillometry analysis was found. Al-Imam (2017) presents a case report of persistently dilated pupils, possibly resulting from multiple and repeated use of psychedelics (Al-Imam, 2017).

There is an elevated need for pupillometric measurement after psychedelic administration, as changes in arousal and state-associated alteration of experience are a key element associated with these drugs. Measuring pupil diameters in humans is indeed a non-invasive way to gain some access to the brain state and neuromodulator circuits (R. S. Larsen & Waters, 2018). Measuring pupil diameter as a response to psychedelic injection, in the rodent model would allow a good insight into the psychedelic-dependent changes in the arousal state of the animal. Should pupil diameter follow the dynamics of EEG data recorded in the previous chapter and the pharmacokinetics of 5-MeO-DMT, such measurements have the potential to be a reliable indicator of a psychedelic-dependent arousal state more applicable in human research and clinical trials. Furthermore, it would allow a direct comparison of the effects of psychedelics between human and animals, as a reliable metric for cross-species comparison is still lacking.

So far, the main technique for pupillometry measurements in animals has relied on using restrained or head-fixed animals (Hughes et al., 2012; Privitera et al., 2020; Semo et al., 2003; Yüzgeç et al., 2018). Restraining rodents allows for a better control of light sources and visual stimulation, but at the cost of spontaneous behaviour and elevated levels of stress which can be a confounding factor in the study of arousal levels. The development of new, reliable, methods of pupil measurement in awake, behaving rodents, akin to the wearable device developed by Carro-Dominguez in humans can lead to less-invasive measurements in both humans and animals. As

pupil size can give an indirect reading of NA levels and arousal, such method can undoubtedly provide new insight on state regulation and arousal fluctuation in freely behaving rodents but would also allow a new and better way of exploring not only spontaneous variations of pupil size in relation to brain states, but also the study of eye movement in freely behaving rodents during wake and sleep state, and more specifically rapid eye movement occurring during paradoxical sleep.

A versatile wearable camera system could also be used to understand how mice navigate their environment depending on different sensory modalities as it could capture not only eye movements, but also whiskers and snout movement, or even potentially any interaction with the head or anterior limbs of the animal. Only a few studies so far reports the use of a wearable, although large, eye-tracking device, suggesting such a method is possible to implement (Meyer et al., 2018; Michaeli et al., 2020).

### 4.1.3 Experimental aims

The main aim of this chapter is to measure changes in attention and arousal processes in freely moving rodents through an established method like the 5-CSRT task, and a novel approach of recording pupil diameters. From the observations made in the previous chapter, we hypothesise that the state induced by an injection of 5-MeO-DMT resulting in increased EEG slow wave activity and decreased theta activity must be associated with a profoundly altered levels of attention and arousal.

To this end, we relied on two separate methods. First, we aimed to set up the technique to measure attention using the touchscreen apparatus with the 5-CSRT task. This first part aimed to measure the alteration of attention in relation to sleep deprivation, paving the way for future experiments with psychedelics. However, our results suggested that this task would not be fit for further analysis with 5-MeO-DMT. The second part focuses on designing and building a device we called oculometre, which is capable of measuring pupil diameter and eye movement in a freely behaving mouse based on a similar device for birds (Ungurean et al., 2021).

With this device, it was possible to record cortical activity in parallel to making video recordings of the eyes following an injection of 5-MeO-DMT. Taken together, the results of this chapter provide novel, less-invasive approaches to monitor the eyes of laboratory mice.

The experimental aims of this chapter are to:

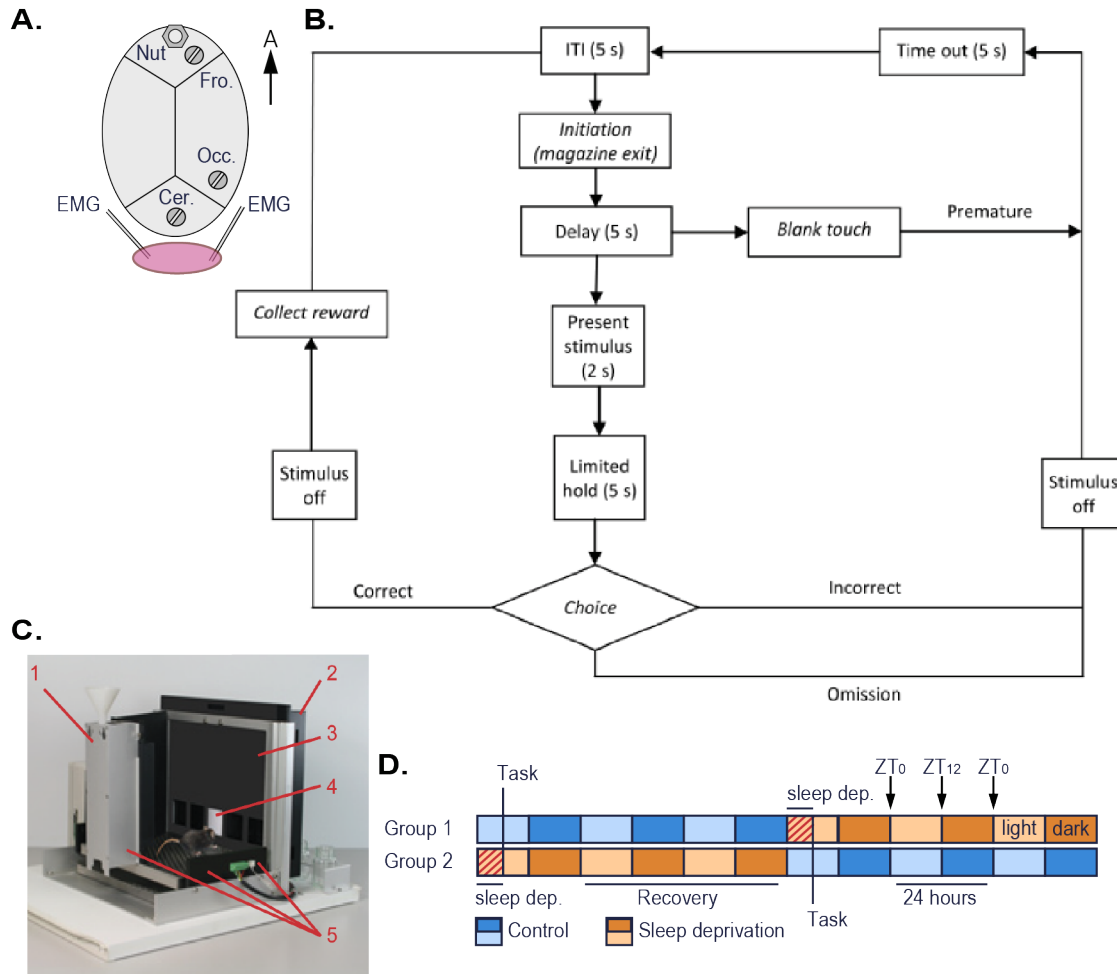
1. Test whether the 5-CSRT task is a viable paradigm to study the effects of sustained wakefulness on attentional performance.
2. Assess the feasibility of designing and building a wearable device to allow pupil monitoring in freely behaving rodents.
3. Measure how 5-MeO-DMT affects pupil diameter and EEG in freely moving rodents.

## 4.2 Methods

### 4.2.1 Animal husbandry

Adult male C57BL/6JOLA-HsdOxuni mice ( $n = 10$ , Oxford Biomedical Science colony, 7 to 13 weeks old) and C57BL/6J mice ( $n = 17$ , Charles Rivers, UK, 7 to 13 weeks old), were kept singly housed in individual Plexiglas cages (20.3 cm x 32 cm x 35 cm) placed inside ventilated sound-attenuated Faraday chambers (Campden Instruments, Loughborough, UK), under a 12-12-hour light-dark cycle (9 am - 9 pm unless otherwise mentioned). The recording room was maintained at  $22 \pm 1$  °C and  $50 \pm 20\%$  humidity. Food and water were provided *ad libitum* throughout the experiment unless the animals were subjected to a behavioural task requiring food restriction protocol as detailed below. All procedures were performed under a UK Home Office Project License and conformed to the Animals (Scientific Procedures) Act 1986.

To investigate the effects of 5-MeO-DMT on pupil size, sleep and wake behaviour, a subset of these animals ( $n = 14$ ) was implanted with a nut (RS components,



**Figure 4.1: Experimental plan of Chapter 4.** **A.** Surgeries. Fro = Frontal. Occ = Occipital. Cer = Cerebellum. The arrow points towards the anterior part of the animal. The EMG are implanted in the nuchal muscle and the nut attached frontally on the skull. **B.** From Mar et al. (2013): “Flowchart overview of the 5-CSRT task. A trial is initiated when the subject enters and withdraws its head from the illuminated magazine. After a 5 seconds delay, a white square stimulus is briefly presented in one of the five response windows. Touching the white-square location either when the stimulus is present (or during a short subsequent limited-hold period) is recorded as a correct trial and rewarded. Collection of reward initiates a 5 seconds ITI. A response in any other response window is recorded as an incorrect trial, and results in a time out period, before the beginning of the 5 seconds ITI. Failure to respond at the screen during the stimulus presentation or the limited-hold period is recorded as an omission and also leads to a time out. Response(s) before the onset of the stimulus is recorded as a premature response, and lead to a time out, but premature trials do not contribute to the session trial count. After the ITI has elapsed, the magazine is illuminated, and the subject can initiate another trial.” **C.** Picture of the operant chamber courtesy of Campden Instruments. 1. Food magazine. 2. Touchscreen. 3. 5-CSRT mask. 4. Stimulus window. 5. Infrared sensors locations. **D.** Experimental plan for the 5-CSRT task. At ZT6, the animals perform in the 5-CSRT after being left alone (Group 1) or a 6-hour sleep deprivation (Group 2). 72 hour later, the conditions are swapped.

DIN 934, M2) on the anterior part of the cranium, among which  $n = 8$  were also implanted with EEG screws in the frontal (AP: +2.0 mm; ML: +2.0 mm) occipital (AP: -3.5; ML: +2.5) and above the cerebellum (2 mm behind Lambda, ML: 0 mm) and EMGs in the nuchal muscle as described in Chapter 2 (Figure 4.1.A).

### 4.2.2 Food restriction

To train the animals in the 5-CSRT task, a total of  $n = 8$  mice followed food restriction protocol. Their weight was measured for three consecutive days and averaged to determine the *ad libitum* free feeding weight (FFW). At the same time, the daily food intake of each animal was measured by monitoring the weight of the remaining food pellets. Differences between consecutive days over a four-day period was averaged to obtain the average food intake of FFW. The target weight in order to motivate the animals to perform in the touchscreen chambers was between 90% and 85% of their FFW (Mar et al., 2013). The target weight was reached by feeding the animals 70% of their average food intake. As the weight was closely monitored, the daily amount of food given to each animal changed based on their weight loss. Once the target weight was reached, 0.2 mL of the reward (Tesco Strawberry milkshake) was added to their diet for habituation prior to them receiving it in the operant chambers.

### 4.2.3 Behavioural paradigm

The 5-CSRT task protocols used a Bussey-Saksida Mouse Touch Screen Chamber Package (80614, Campden Instruments). The chambers were trapezoidal, black-walled boxes of 20 cm high, 18 cm length and 24 cm to 6 cm wide with a plexiglass panel floor of 18 cm x 24-6 cm, equipped with infrared sensors and a camera on top to monitor the activity of the animals. A magazine for delivering liquid reward was fitted to the chamber, equipped with its own light and infrared sensor system (Figure 4.1.B). The box was also equipped with lights, a tone generator, and a

click generator. Each training stage was realised using the ABET II software for touchscreen apparatus (Campden Instruments) and corresponds to a Campden Instruments premade software setup controlling the lights, food dispenser, timings, and the random stimulus appearance. The animals were trained through the different stages of the task as advised by Mar et al. (2013) (Figure 4.1.C)

Stage 1 was designed to train the animals to use the touchscreen and was subdivided as follows:

- a) The mice were placed within the environment with the 5-CSRT task setup, including the placement of a 5-hole mask where the stimuli of later tasks will occur, thus allowing a clear separation between visual targets. The screen was kept lit but black as no visual target was displayed and the magazine full of milkshake. Each session lasted one hour. If all the milkshake was consumed from the magazine during the session, the mice moved to the next step. If the mice failed to consume all milkshake, the session was repeated the next day.
- b) In this stage, the visual target stimuli were presented. The stimulus consisted of a plain white square located in one of the squares of the screen mask. One stimulus was presented for 30 seconds, and, at the end of the stimulus, 1 drop of milkshake was delivered to the magazine. 5 seconds after the reward was delivered, a new stimulus was presented at another random location. Should the animal touch the screen during the stimulus presentation, the stimulus disappeared, then the magazine lighted up, a 3kHz tone was played, and a reward triple the size of the usual reward was delivered. 5 seconds after the mice collected the reward, another trial started with the presentation of a stimulus at another random location. The session ended after 30 trials or 60 minutes. This stage was repeated once a day until the animal performed 30 trials.
- c) In this stage, the mice had to touch the stimulus for it to disappear. Once the stimulus was touched, 1 drop of reward was attributed. The stimulus



appeared on another random location 5 seconds after reward delivery. If the mice did not respond or touched the screen at a location without a stimulus, no reward was delivered, and the stimulus remained on the screen. Each session ended after 30 trials or 60 minutes. Once 30 trials were completed, the mice moved to the next stage. This stage was repeated once a day until the animal performed 30 trials.

- d) In this stage, the mice had to start a trial by poking their nose in the magazine when its light is turned on. Once realised, the light went off and a click sound was played for 0.2 seconds. The stimulus was presented immediately after the end of the click sound and the mice had to touch the screen to receive 1 drop of reward. 5 seconds after reward collection, the magazine light was turned on and the mice had to poke their nose in to initiate the next trial, with a stimulus location assigned at random. The sessions stopped either after 60 minutes or when 30 trials were completed, whichever came first. This stage was repeated once a day until the animal performed 30 trials.
- e) This stage was designed to give negative feedback if the touchscreen is touched but not where the stimulus is located. The same task as in d) were performed. If the mice poked the touchscreen at a non-stimulus location, the stimulus went off and a 5-second time out period with the house light on started. After this time out period, the animal could start the next trial by poking its nose into the magazine. On the trial following a mistake, the stimulus was still located at the same position as in the mistake trial. To proceed to the next stage, the mice were expected to have a success rate above 75% within 35 minutes on 2 consecutive sessions. If a success rate below 75% was observed, the mice had to perform this task on the next day. This stage concluded stage 1.

Stage 2 was designed to train the animals to perform in the 5-CSRT task and was subdivided as follow:

- a) The 5-CSRT task is described in Figure 1.C and contained three new task components. First, a 5-second delay was introduced after the initialisation of a trial, once the animal removed its nose from the magazine as introduced in stage 1. Second, the stimulus now disappeared 8 seconds after it appeared at a random location. Finally, the animal has 5 more seconds to touch the screen at the right location after the disappearance of the stimulus. A trial was labelled “premature” if the animal poked the screen in the first 5-second hold period prior to stimulus presentation. A trial was labelled “incorrect” if the mice poked the screen at the wrong location. A trial was labelled as an “omission” if the animal failed to touch the screen during the stimulus presentation or during the 5 seconds after the stimulus terminated. All of these trial-types (i.e. premature, incorrect or omission) were followed by a 5-second time out where the house lights were turned on. A trial was labelled “correct” if the animal touched the screen during stimulus presentation or during the 5 seconds after the stimulus terminated. After a correct trial, the animal received 1 drop of reward and, if the stimulus was immediately turned off if it was still present. Each trial was then followed by a 5-second inter-trial interval after which the animal must poke the magazine to start a new trial. Response accuracy was measured as the percentage of correct response amongst the trials not including omissions and premature responses. The sessions stopped either after 60 minutes or when 50 trials were completed, whichever came first. Animals were trained daily, at least 5 times per week. To proceed to the next training stage, the mice were expected to have a response accuracy of above 80% and trial omissions represented less than 20% of the trials for 3 out of 4 sessions.
- b) This stage reduced the stimulus duration to 4 seconds. The task and the requirements to move to the next sessions were the same as before.
- c) This stage reduces the stimulus duration to 2 seconds. The task and the requirements to move to the next sessions were the same as before. The mice

remained at stage c) until all mice from the cohort reached and passed stage c).

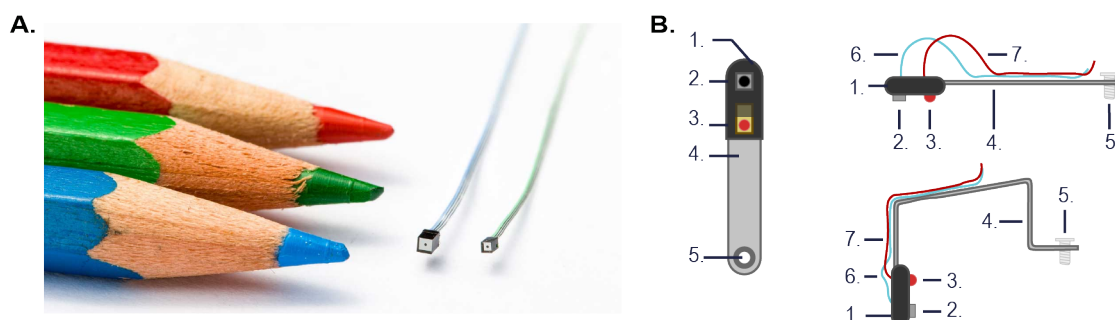
In order to assess the effects of sleep deprivation, the 5-CSRT task protocol followed the same steps as provided by Campden Instruments with their touchscreen apparatus and ABET II software, as also described in Mar et al. (2013) (Figure 4.1.C) The task was the same as in stage 2.c of the training above. Each animal performed the test 3 times, one as a baseline, one after sleep deprivation, and one without sleep deprivation. Each testing session were separated by 3 days and the animals performed after or without sleep deprivation in a counterbalanced, random order.

#### **4.2.4 Sleep deprivation**

To monitor the effect of sleep deprivation on attention, the animals ( $n = 8$ ) were sleep deprived for 6 hours using the technique described in Chapter 2. The nest was removed from the home cages and novel objects were presented at regular interval. Sleep deprivation occurred from light onset (9 am; ZT = 0) until ZT6 (3 pm) and was immediately followed by behavioural testing (Figure 4.1.D).

#### **4.2.5 Oculometry**

To monitor levels of arousal, we built a device called an oculometre consisting of an aluminium holder with a miniature camera directed at the eye (NE2D B&W V90F2.7\_2m, NanEye, Austria) and a far-red LED (Luxeon Rebel LED 720nm LXML-PF-1, Luxeon Star Leds, Canada, Figure 4.2, maintained together with Sugru mouldable silicone rubber (Black, Sugru, Germany). The LED was powered through a 700 mA BuckPuck DC driver (3023-D-E-700, Luxeon Star Leds) and connected to the BioAmp Processor (RZ 2, Tucker-Davis Technologies) with an AV 9-way cable (6126423, RS Components) for activation through user input. The camera was connected to a PC via a wired board (985-NanoUSB 2.2, Mouser UK)



**Figure 4.2: Details of the oculometre.** **A.** NaNeye camera used for the crafting of the oculometre. Image from Mouser website. **B.** Schematic of the oculometre (not to scale) facing view (left), profile view (right) unfolded (top) or folded and ready to be attached (bottom). 1. Sugru paste. 2. Camera. 3. LED. 4. Aluminium plate. 5. Screw. 6. Optic fibre. 7. Insulated copper cable.

and the video recorded with the NanEye Viewer software (NanEye, v6.0.3, default configuration) for NanEye 2D and NanoUSB2 board at a frame rate between 45 frame per second (fps) and 55 fps. Exposure, gain and offset were automatically calibrated through the software to minimise loss of data due to poor lighting conditions which can sometimes occur during some behaviours such as digging or nest building. The device was secured to the head of the mouse with a slot cheese nylon machine screw (M2 x 6 mm, DIN 84, RS Components) adjusted to fit a stainless steel hexagonal nut (M2, DIN 934, RS Components) fixed to the skull with dental cement SuperBond (Prestige Dental Products Ltd, Bradford, UK). Taken together, the oculometric device costs can be rounded to £150 for a weight below 500 mg, with a one-time £400-900 purchase of a wired board.

To test the oculometre, the first recorded images were performed under anaesthesia ( $n = 3$ ). The videos of freely moving mice ( $n = 14$ ) were recorded following one 1-hour session of habituation, to the device during which all mice were fitted with their respective oculometre, and the arms oculometre angled to record the most optimal view of the eye. During recording days, all animals wore the device at least 10 minutes before the start of experiments. The recordings started at light onset and were followed by the injections of either vehicle or 5-MeO-DMT as described below. As the device is quite cumbersome, and could impact normal behaviour of

the animals, it was decided to only record 60 minutes of video per subject per session.

### 4.2.6 Injections

To monitor the effects of 5-MeO-DMT on pupil size, the mice ( $n = 14$ ) underwent a pseudorandom and counterbalanced crossover design as detailed in Chapter 2 in which they received an injection of either vehicle (5 mL/kg saline) or 5 mg/kg 5-MeO-DMT (in 1 mg/ml saline, Beckley Psytech) at light onset ( $ZT = 0$ ). A subpart of these animals ( $n = 8$ ) was also used for subsequent experiments which will be discussed in Chapter 6.

To test the oculometre and correctly plan the experiment in freely-behaving mice, a group of animals ( $n = 3$ ) received their injections of vehicle and 5-MeO-DMT under 1.5 – 2% Isoflurane anaesthesia with an oxygen flow rate of 2 L/min while the animal was head-fixed to a stereotaxic frame (David Kopf Instruments, California, USA; Model 900 Small Animal Stereotaxic Instrument) and resting on a heat pad. To assess whether the oculometre would monitor putative pupil mydriasis in anaesthetised animals, two injections were performed one hour apart, and their respective effects were recorded for 1 hour. The injections were either vehicle or 5-MeO-DMT at the same concentrations as stated above, injected in a counterbalanced order as detailed in Chapter 2.

### 4.2.7 Analysis

Video recordings were first converted into AVI format and analysed using DeepLabCut (Mathis et al., 2018; Nath et al., 2019). The model trained for scoring the pupil used the following parameters: resnet = 50, Shuffle 6; 5000000 trials. Pupil diameter was calculated based on the coordinate of all scored points as the average distance between opposite points. Further details on the scoring and calculation are made in subsection 4.3.3.

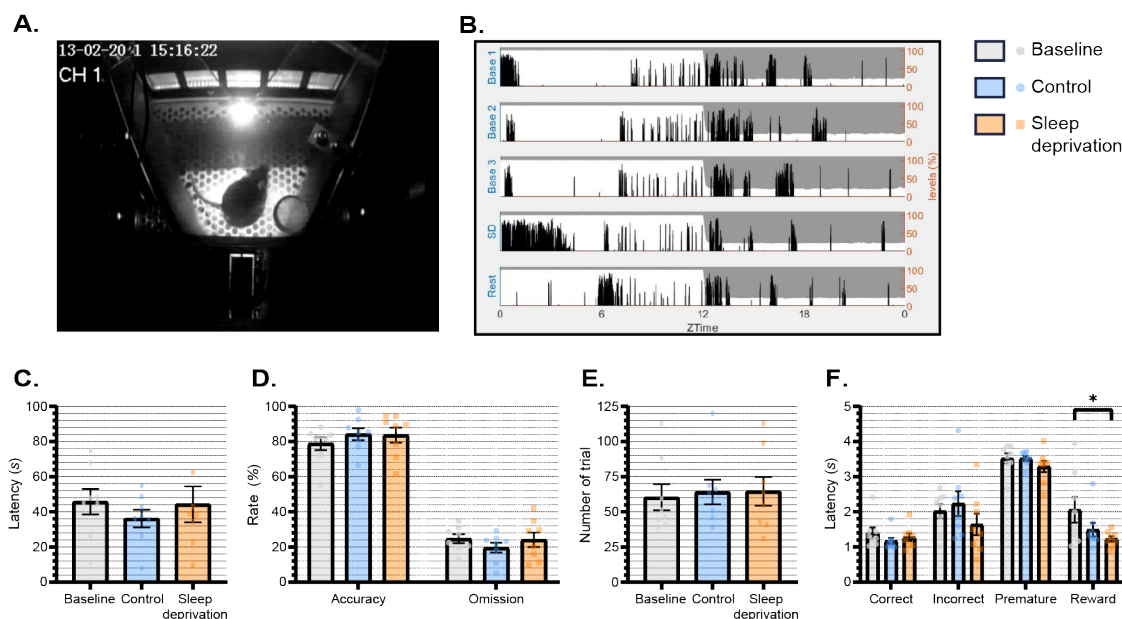
All data analysis presented in this chapter were processed in MATLAB (The MathWorks Inc., Natick, MA, USA) and analysed using MATLAB for producing the analysis tables, and then in Prism to compute the statistical analyses. (GraphPad Software, Boston, Massachusetts USA, version 10.2.0 (392)). All data were tested for normality prior to analysis using a Shapiro-Wilk test. Statistics for the 5-CSRT task were performed with a repeated measures (RM) ANOVA or Friedman's test with Dunn's test as a post-hoc as a non-parametric equivalent. The analysis of EEG spectra was conducted on log-transformed values and compared with mixed-effect (ME) analysis and uncorrected Fisher's LSD post-hoc tests. Time course of EEG activity and pupil size were also analysed with a ME analysis and uncorrected Fisher's LSD, and the correlations between EEG activity and pupil size calculated using Pearson's correlation. The Greenhouse-Geisser (GG) correction was applied when sphericity of the data was not found. The results of the statistical analyses are detailed in Appendix B.

## 4.3 Results

### 4.3.1 Sleep deprivation does not alter attention

To investigate the impact of sleep deprivation on sustained attention, the mice were trained to use the touchscreen apparatus as recommended by Mar et al. (2013) (Figure 4.3.A). Two rounds of behavioural testing were made with the same cohort of animals. In the first period of behavioural testing, their bodyweights were kept close to 85% of FFW. However, the sleep deprivation protocol failed as the control animals stayed awake as well. This likely reflected increased arousal as a result of the anticipation of food reward during subsequent performance of the behavioural task.

After this first attempt, the animals were fed *ad libitum* until they achieved a stable bodyweight for three consecutive days before performing the experiment again. This new bodyweight was considered their free-feeding body weight and the target weight for food restriction was adjusted accordingly. Given that this cohort of mice had already been trained on the task, the animals reached their learning plateau



**Figure 4.3: Effects of sleep deprivation on attention.** **A.** Frame from the video recording monitoring the behaviour of the animal. Light is on as the animal performed an “omission” trial, incidentally, allowing for a clearer view of the test chamber. On top, the touchscreen is fitted with a 5-windows mask. The animal initiate a trial and gets the rewards by the rectangle at the bottom of the image. **B.** PIR data showing the activity of one representative animal during the light (ZT0 to ZT12) and dark (ZT12 to ZT0) period over the recording and testing days. The white background indicated the light intensity levels. The black bars represents activity. **C.** Latency to the initialisation of a new trial by the animal, comparing baseline, control, and sleep deprivation data. **D.** Accuracy and omission rates between all conditions expressed in percentage of trials across the session. Accuracy is calculated as the proportion of correct trials across all trials where the animal engaged with the screen. Omission is calculated as the proportion of omission trials across all trials of the session. **E.** Total number of trials for all conditions. **F.** Latency to correct, incorrect, or premature screen touch by the animal, and latency to reward collection. Data are presented as mean + SEM (\* $p < 0.05$ ).  $N = 8$  for panels C-F. See Table B.1 for statistical analyses.

much faster, potentially allowing for a lighter food restriction (at approximately 90% of their *ad libitum* feeding bodyweight). PIRs activity monitoring systems were used to monitor the locomotor activity of each animal throughout the experiment during training, testing and recovery days, demonstrating a successful sleep deprivation protocol (Figure 4.3.B).

The animals were woken up 10 to 15 minutes before the test during the transport of their home cage to the test chamber. During behavioural testing, all animals,

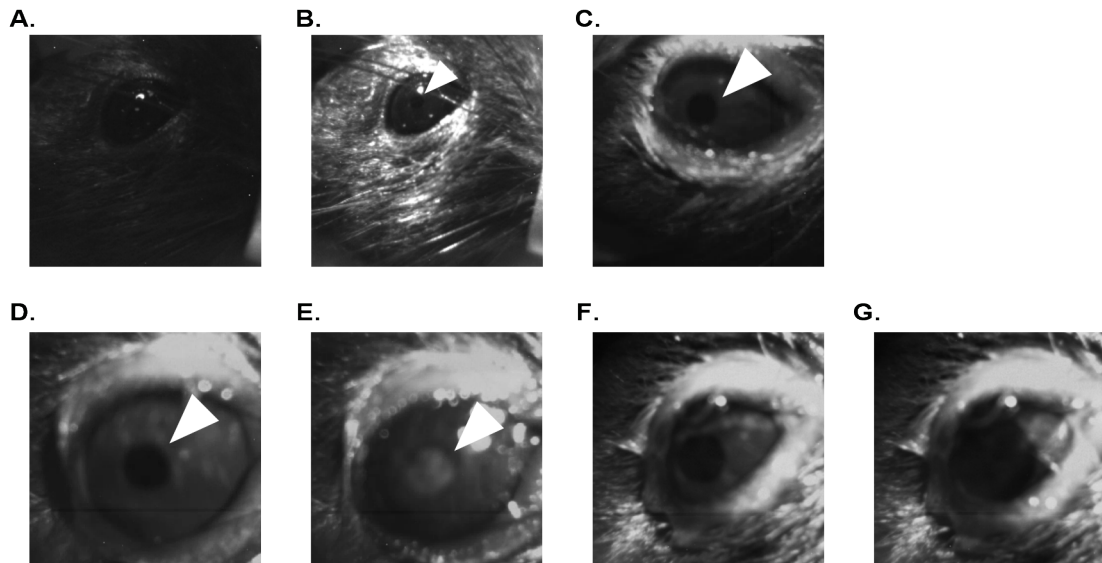
for all of the experimental conditions, initiated trials on average within 35 seconds to 45 seconds.

There was no significant difference between the three conditions for latency to initiate trials (RM-One-way ANOVA, effect of condition:  $F_{2,14} = 0.79$ ;  $p = 0.47$ ; Figure 4.3.C). Choice accuracy was always between an average of 75% and 8% correct, above the 20% chance levels of performance (Robbins, 2002), and didn't differ between conditions (Friedman's test,  $\chi^2(2) = 5.25$ ;  $p = 0.079$ ), with the omission rate between an average of 18% to 25% which again didn't differ between the three conditions (RM-One-way ANOVA, effect of condition:  $F_{2,14} = 1.14$ ,  $p = 0.35$ ; Figure 4.3.D), with a stable total amount of trials of around 60 trials per sessions for all three conditions (Friedman's test,  $\chi^2(2) = 0$ ;  $p > 0.99$ ; Figure 4.3.E).

It was found that the animals performed similarly, and the performance did not change across sessions. On average, all animals showed the same latencies across all three conditions or touching the correct part of the screen, with an average between 1.1 and 1.4 seconds (Friedman's test,  $\chi^2(2) = 5.25$ ,  $p = 0.079$ ), and for touching an incorrect hole with no stimulus, with an average between 1.6 and 2.3 seconds (RM-One-way ANOVA, effect of condition:  $F_{2,14} = 0.88$ ,  $p = 0.43$ ) (Figure 4.3.F). A premature response occurred on average between 3.1 and 3.6 seconds after trial initiation and this latency measure didn't differ between conditions (RM-One-way ANOVA, effect of condition:  $F_{2,14} = 0.90$ ,  $p = 0.43$ ). The only significant difference between conditions was found in the latency to collect the reward, as the sleep deprived animals had a shorter latency on average ( $1.23 \pm 0.078$  s) compared to baseline ( $2.060 \pm 0.38$  s) (Friedman's test,  $\chi^2(2) = 6.25$ ,  $p < 0.05$ ; Dunn's post hoc  $p < 0.05$ ), but not compared to their control condition (Figure 4.3.F).

The PIR data shows that sleep deprivation was successful, with the animals remaining inactive during the recovery days (Figure 4.3.B). PIR data also shows that the animals remained active during most of the light period, after performing, and were largely inactive in the second part of the dark period over several days (Figure 4.3.B). It is likely that food-driven excitation entrained the animals and kept them awake. In Chapter 3, the presentation of a sugar pellet to animals after





**Figure 4.4: Pilot observations of the pupil in anaesthetised animals.** **A.** Prior to crafting the device, the camera was brought to the surgery room and recorded anaesthetized animals under ambient light. The reflection of the ambient light source is visible as white spot on the eye of the animal. **B.** The same animal, after pointing surgical light directly at the eye. **C.** On an anaesthetized animal, with the oculometre crafted and placed next to the animal, illuminated by the LED. **D.** protective gel was applied to protect the eye, artificially increasing the size of the eye and allowing a clearer recording. **E.** The same animal as C and D after one hour of recording showing signs of dryness of the eye. **F.** A mouse after anaesthesia with eye-protection gel immediately following an injection of 5-MeO-DMT. **G.** The same mouse as in F. 2-5 minutes after the injection of 5-MeO-DMT showing sign of pupil mydriasis.

an injection of 5-MeO-DMT suggested that 5-MeO-DMT had anhedonic properties, therefore it would have been highly probable that the animals, once injected with 5-MeO-DMT would have performed poorly in the 5-CSRT task due to a lack of interest in the reward, rather than an alteration of attentional processes. Because of these two reasons, it was decided that the 5-CSRT task would not be fit to monitor the putative alteration of attention induced by 5-MeO-DMT.

### 4.3.2 The development of the oculometre

With Chapter 3 suggesting the occurrence of sleep-like slow wave activity during wakefulness in the presence of 5-MeO-DMT, we hypothesised that 5-MeO-DMT might alter the levels of arousal. As studies suggest that pupillometry allows a read

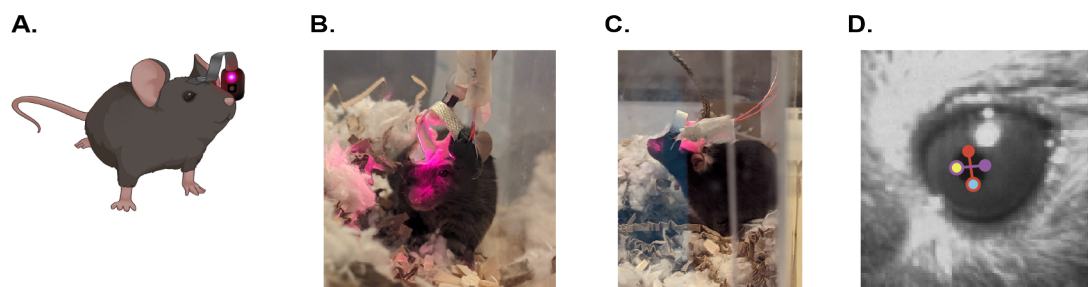
out of the levels of arousal in rodents (Yüzgeç et al., 2018), we decided to develop a novel apparatus to monitor pupil diameter in freely moving animals, thus allowing a read out of arousal levels during spontaneous behaviour.

Firstly, to determine whether the micro-camera was a viable approach to measure pupil size in mice, we performed several 5-minutes recordings of human eyes, panoramic views of several rooms including the surgery room, a bedroom, and an office space, as well as multiple recordings of a moving black dot on a white background. We then realised several other 5-minutes recordings of the eyes of an anaesthetised animal during which it was determined that a light source would be necessary to perform high quality recording (Figure 4.4.A-B). A 720 nm LED was selected in order not to elicit any light-dependent constriction reflex of the pupil, and a handling device composed of an aluminium arm capable of holding the LED and camera was crafted based on prior work with monocular light stimulation conducted by Jose Prius-Mengual, and we named the holder “oculometre” for future reference (Figure 4.2.B).

To test whether the oculometre would be capable of recording longer sessions without overheating, subsequent recordings were made on anaesthetised animals for several hours, with the oculometre placed next to the animal but not attached to it. Tactile inspection indicated that the device was not overheating for the entire duration of the recording session, suggesting the plausibility of long-term recordings.

The pupils of all mice were visible, but only for a short period of time before the eye would show signs of dryness, showing a growing white spot that would cover the pupil if not most of the eye (Figure 4.4.C-E). To counter these effects, Viscotears drops were immediately applied, also allowing for a clearer visualisation of the pupil (Figure 4.4.F).

5-MeO-DMT was then injected in the anaesthetised animals and reliably induced a visible and durable pupil mydriasis in all animals, which suggested an effect would be observed in freely behaving animals (Figure 4.4.G). Small variations of the pupil size were noted throughout the recordings. When the room lights were turned



**Figure 4.5: Representative visualisation of the oculometre.** **A.** Schematic of a mouse wearing the oculometre. **B-C.** Photographs of mice wearing the oculometre with EEG implants. **D.** Sample image from the oculometre software with the points manually scored at the north- (red), east- (purple), south- (cyan) and west- (yellow) most location of the pupil. The North-South vector (red line) and East-West vector (purple line) used to calculate the pupil size are also represented.

on either by accident or to retrieve some materials, it was also observed that the pupillary reflexes of the animals under anaesthesia did not seem lost as the pupil showed brief constriction when the lights were turned on.

The LED and camera were then fixed to the aluminium frame selected specifically for its malleability, allowing an easy manipulation of the camera angle, and its weight, which was light enough for the animals to wear (Figure 4.2.B).

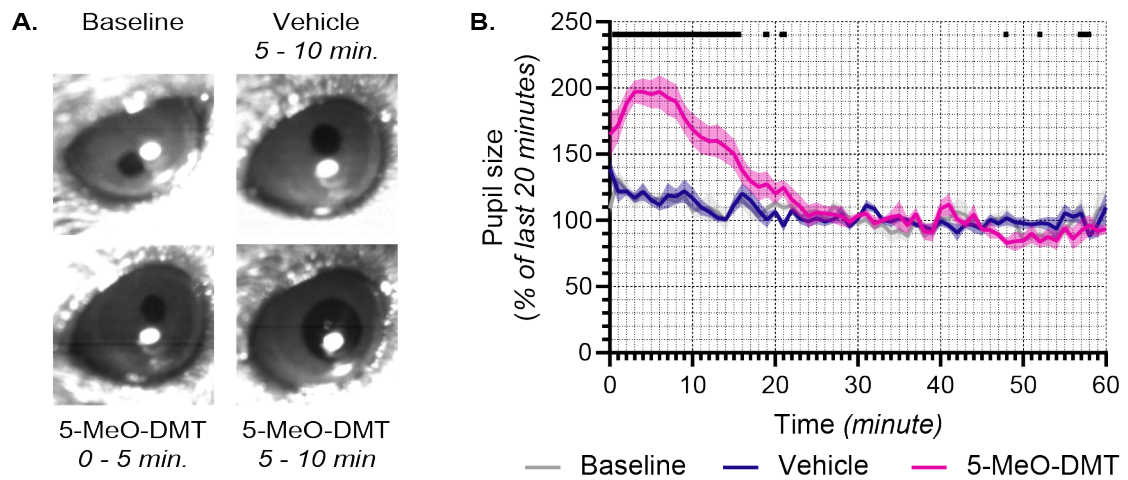
### 4.3.3 5-MeO-DMT injections induces pupil mydriasis in freely moving mice

The subsequent recordings were performed with the oculometre attached to a freely moving animal (Figure 4.5.A-C). Ample habituation time to the recording device was given to the mice, as it was suspected that having the oculometre in front of their eyes might be bothersome. The mice habituated quite quickly to the device, rapidly exhibiting normal nesting and grooming behaviour. With these behavioural observations, subsequent experiments always included a few hours of habituation to the oculometre the day prior the recording sessions, and 10 minutes habituation sessions just before the actual recordings. The oculometre proved itself to be sturdy, as scratching behaviour did not move the device in its entirety nor the camera.

Clear video recordings were acquired. The versatility of the device also allowed us to monitor the anterior-most part of the mice, including whiskers and snout. A striking observation during these habituation days was made when a novel object was placed in the environment. Indeed, some camera placement allowed for a visualisation of the whiskers. It was noted that, when approaching a novel object, the pupils were often placed in the posterior part of the eye and not toward the object, while the whiskers were oriented frontally. This would support the idea that the mice do not primarily rely on their visual system to explore their environment (Michaël et al., 2020). The pupils of all animals were also very dynamic, showing constant changes in size and position.

After the recordings, the videos were converted into an .avi format offline using the built-in conversion tool. Using DeepLabCut build-in user interface, the locations of the pupil for a random sample of frames from all videos of all animals were scored. In total 400 frames were annotated. Four points were used to track the pupils: the northernmost, the southernmost, the easternmost and westernmost points. Each point was marked only if it was clearly in view and not hidden by the eyelid or any light artefact. Once scored, the DeepLabCut model was trained on a randomly generated sample of images. The trained model then automatically scored all videos, and only points labelled by the model with a confidence above 99% were kept. Pupil diameter was estimated as the average of the distance between the Northern and Southern point and the distance between the Eastern and Western point.

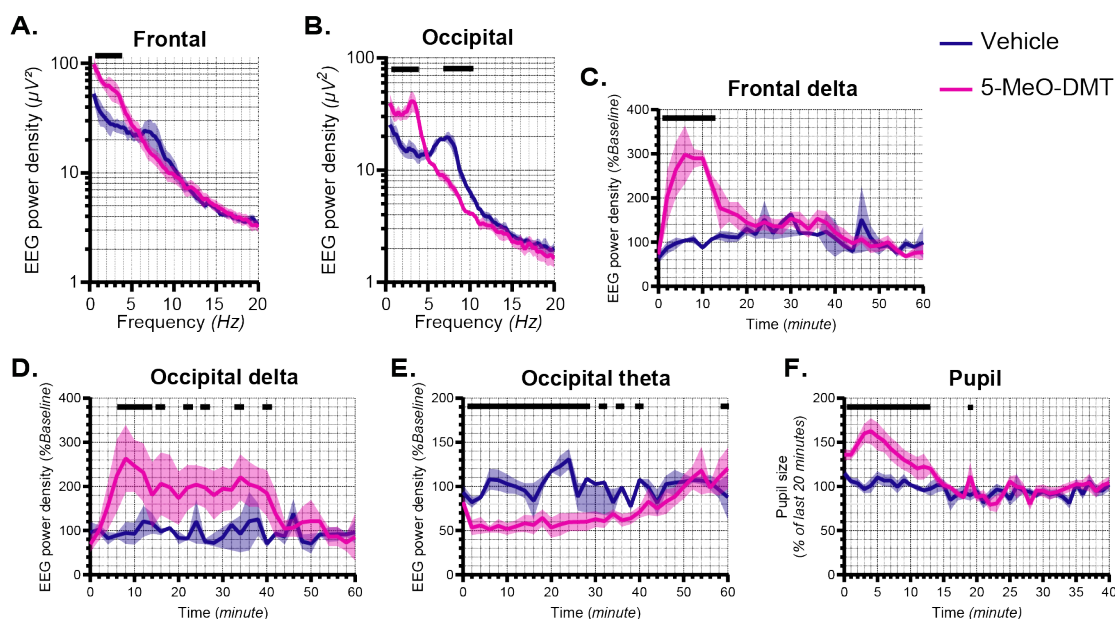
The animals were then injected with vehicle or 5-MeO-DMT to measure the pupillary response and recorded for 1 hour per session, with each session separated by 3 days of recovery. As the placement of the oculometre can change between session, pupil sizes are expressed as a % of the last 20 minutes of the 1-h recordings. Following a vehicle injection, the pupil size was indistinguishable from baseline except in the first minute where it was found that it was slightly more dilated (ME analysis, time point\*condition:  $F_{120,422} = 9.32$ ,  $p < 0.0001$ ; Fisher's LSD  $p < 0.05$ ) (Figure 4.6.A).



**Figure 4.6: Effects of 5-MeO-DMT on pupil diameter alone.** **A.** Representative images of baseline (top left), within 5-10 minutes following an injection of vehicle (top right), immediately following the injection of 5-MeO-DMT (bottom left), 5-10 minutes after the injection of 5-MeO-DMT. **B.** Time course of pupil diameter over 60 minutes following an injection of 5-MeO-DMT in  $n = 6$  mice. Data are presented as mean + SEM. Black horizontal lines denote a significant difference between 5-MeO-DMT and vehicle condition (Fisher's LSD  $p < 0.05$ ). See Table B.2 for statistical analyses.

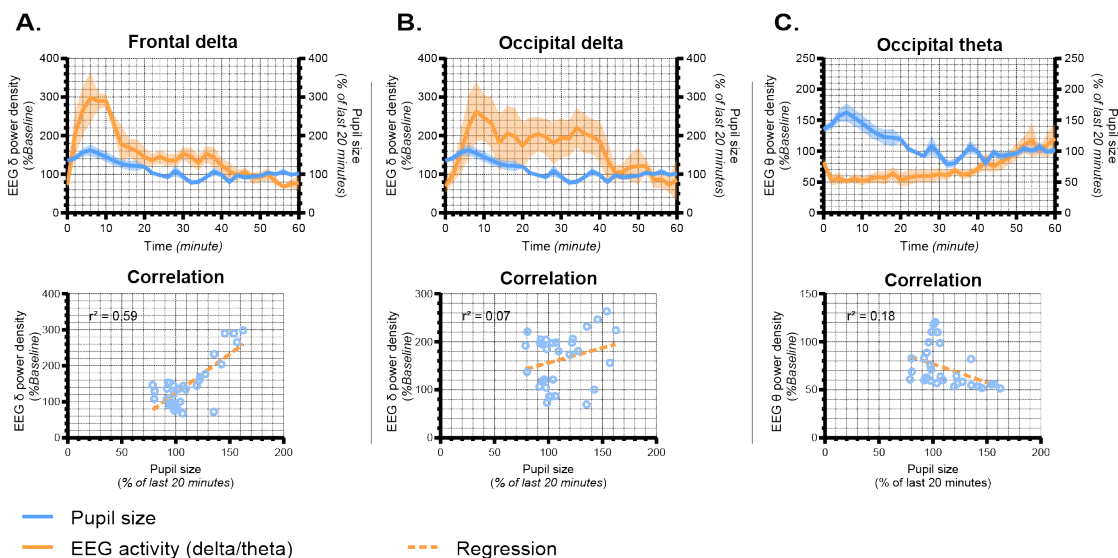
5-MeO-DMT however, elicited a pupil dilation of about 220% of its baseline size within 5 minutes following the injection, and thus was significantly more dilated than in the baseline or vehicle conditions (Fisher's LSD  $p < 0.05$ ) (Figure 4.6.A-B). By plotting the time-course of pupil diameter, the increase in pupil size started immediately after the injection, peaked 5 minutes after the injection, and then returned to baseline 15 to 20 minute after the injection (Fisher's LSD  $p < 0.05$ ) and while no increase in pupil diameter was observed after an injection of vehicle.

The same video-recordings were then performed in mice with EEG implanted above the frontal and occipital cortices. After scoring and retrieving the artefact-free episodes of wake following the injection, the EEG results reported in Chapter 3 were replicated, with a significant increase of slow wave activity in both frontal and occipital derivation, and a suppression of the theta activity in the occipital derivation (ME analysis, Frontal: frequency\*condition:  $F_{78,75} = 13.22$ ,  $p < 0.0001$ ; Occipital: frequency\*condition  $F_{78,76} = 9.28$ ;  $p < 0.0001$ ) (Figure 4.7.A-B).



**Figure 4.7: Effects of 5-MeO-DMT on EEG and pupil diameter.** A-B. EEG spectral power in the frontal (A.) and occipital (B.) derivation after an injection of 5-MeO-DMT or vehicle during the episodes of wakefulness from 0 to 30 minutes post-injection. C. Time course of the frontal delta activity during wake episodes after an injection of 5-MeO-DMT or vehicle. D. Time course of the occipital delta activity during wake episodes after an injection of 5-MeO-DMT or vehicle. E. Time course of the occipital theta activity during wake episodes after an injection of 5-MeO-DMT or vehicle. F. Time course of the pupil diameter size in wake episodes after an injection of 5-MeO-DMT or vehicle. Data are presented as mean + SEM. Black horizontal line denotes a significant difference between 5-MeO-DMT and vehicle (Fisher's LSD  $p < 0.05$ ).  $N = 8$  for all panels. See Table B.3 & Table B.4 for statistical analyses.

A time course analysis of the frequencies of interest showed that in the frontal derivation, the EEG activity in the delta range was initially significantly increased after an injection of 5-MeO-DMT, suggesting a decrease in cortical arousal (ME analysis, time point\*condition:  $F_{30,7} = 5.19$ ,  $p < 0.05$ ; Fisher's LSD  $p < 0.05$ ; Figure 4.7.C). As the effects of psychedelics are known to change with the environment, observing results as in Chapter 3 suggests that the oculometre and LED did not seem to alter the altered state induced by 5-MeO-DMT. However, the frontal delta activity increased for 10 to 15 minutes as opposed to the 40 minutes observed in Chapter 3, suggesting some degree of alteration. In the occipital derivation, the EEG activity was increased in the delta range between 5 and 15 minutes and remained relatively high, showing an average increase of about 200% for 40 minutes (ME



**Figure 4.8: Correlations between EEG activity and pupil diameter.** **A.** Time course of frontal EEG delta activity power and pupil size (left) and correlation of frontal EEG delta activity power and pupil size with regression line (Pearson  $r^2 = 0.59$ ; right). **B.** Time course of occipital EEG delta activity power and pupil size (left) and correlation of frontal EEG delta activity power and pupil size with regression line (Pearson  $r^2 = 0.07$ ; right). **C.** Time course of occipital EEG theta activity power and pupil size (left) and correlation of the occipital EEG theta activity power and pupil size with regression line (Pearson  $r^2 = 0.18$ ; right). Data are presented as mean + SEM. Black horizontal line denotes a significant difference between 5-MeO-DMT and vehicle (Fisher's LSD  $p < 0.05$ ).  $N = 6$  for all panels. See Table B.5 for statistical analyses.

analysis, time point\*condition:  $F_{30,15} = 2.98$ ,  $p < 0.01$ ) (Figure 4.7.D). Occipital EEG activity was suppressed in the theta range following a similar trend as the delta activity (ME analysis, time point\*condition:  $F_{30,6} = 5.20$ ,  $p < 0.001$ ; Fisher's LSD  $p < 0.05$ ; Figure 4.7.E). Both occipital delta and theta activity followed similar trend as the one observed in Chapter 3. We replicated our previous observation on pupil diameter and saw a significant increase in pupil diameter following an injection of 5-MeO-DMT within 30 minutes of the injection (ME analysis, time point\*condition:  $F_{40,121} = 3.43$ ,  $p < 0.0001$ ; Figure 4.7.F).

By following the time-course of delta and theta activity and comparing them to the time course of pupil size, it was possible to correlate frontal delta activity dynamics to pupil size (Pearson's simple linear regression,  $r^2 = 0.59$ ,  $p < 0.0001$ ), as both peaked within 5 minutes and returned to vehicle-comparable levels within



15 to 20 minutes (Figure 4.8.A). However, no correlation was found between the occipital delta activity and pupil size (Pearson's simple linear regression,  $r^2 = 0.07$ ,  $p = 0.16$ ) (Figure 4.8.B), and it was found that occipital theta was anticorrelated with pupil size (Pearson's simple linear regression,  $r^2 = 0.18$ ,  $p < 0.05$ ) (Figure 4.8.C).

## 4.4 Discussion

### 4.4.1 The monitoring of attention in mice

The measurement of attention with reliable tools such as touchscreen-dependent paradigms can allow cross-species comparison of performance under the influence of psychoactive substances or after sleep deprivation paradigms such as sleep deprivation (Carli et al., 1983). However, it was not possible to come to any meaningful conclusion regarding the effects of sleep deprivation on attention, besides that sleep deprived animals seemed to retrieve their food faster.

Sleep deprivation was conducted successfully in the second phase of the experiment, as suggested by the PIR data. However, the PIR data also revealed a shift in the sleep/wake cycle, with long periods of inactivity happening during the dark period and long periods of activity following training and feeding in the light period. It was clear that the animals were entrained by the touchscreen and food deprivation paradigm which were potentially giving stronger cues than light changes, and therefore it is entirely plausible that the sleep deprived animals had a few hours of rest before the sleep deprivation, potentially nullifying the results.

This was the second attempt to perform such an experiment, with sleep deprivation having failed in the first attempt. A suggested solution was to perform training and feeding at a different, random time of day, to limit the excitation induced by feeding and rewards. Indeed, it has been found that VTA neuron firing is correlated to salient stimuli increasing arousal levels and delaying SWS (Eban-Rothschild et al., 2016). However, it is highly probable that this method would have induced more variability in terms of motivation. Another solution would have been to feed the animals at or after dark onset every day.



It was also noted that some food restricted animals showed an overall poor engagement with the task itself, with an elevated time to initiate trial. This could be an illustration of an overall poor motivation to perform. All animals were trained following the recommended procedures (Mar et al., 2013). The engagement was high enough according to these recommendations, so we do not think the reward was the cause. As previous studies have reported that mice poorly engage with the 5-CSRTT (Carli & Samanin, 1992; Koskinen et al., 2000a, 2000b; Mar et al., 2013), this might be a limitation of the task itself. It has been shown that in some cases mice will exhibit a reduced level of performance if stress levels are too high or too low (Yerkes & Dodson, 1908). It is plausible that the animals are not stressed enough and have no incentive of participating, or that they are in fact too stressed, either by the system or the food restriction paradigm, and do not engage with the task because of that.

Administration of psychedelics has also been shown to decrease performance in the 5-CSRT task (Carli & Samanin, 1992; Koskinen et al., 2000a, 2000b; Robbins, 2002). As mentioned in the introduction of this chapter, this decrease has been linked to altered 5-HT neurotransmission. Carli and Samanin (1992) report that the alteration of 5-HT<sub>2</sub>-associated neurotransmission, as opposed to the 5-HT<sub>1</sub>-associated effects, affect response latency and omission rate without impairing food motivation or motor control.

Studies on the 5-HT<sub>2</sub> class of receptors suggest their involvement in impulsive behaviour, with several 5-HT<sub>2A</sub> antagonist such as ketanserin and ritanserin blocking impulsive behaviours, but not 5-HT<sub>2C</sub> antagonists, with no effect on motor activity (Koskinen et al., 2000a, 2000b). It was also reported that pindolol, a 5-HT<sub>1A</sub> agonist did not elicit any effect on the impairment of attention and impulse behaviour induced by SSRI, suggesting a stronger involvement of 5-HT<sub>2A</sub> receptors (Wingen et al., 2007, 2008).

Although not conducted in this chapter, the measurement of acute attentional changes induced by psychedelics would provide interesting results regarding global brain physiology and a combined experiment with a behavioural task and EEG recordings following an injection of 5-MeO-DMT could help understand the consequences of a global increase of slow wave brain activity and suppression of theta activity on attention and impulsive behaviour.

Psychedelics have also been reported to enhance DMN activity, an argument in favour of the feeling of disconnection induced by altering 5-HT neurotransmission (Mason et al., 2020). Such a prospect should have paved the way for a 5-CSRT task led study with 5-MeO-DMT and electrophysiology. However, because of the first failure of the sleep-deprivation experiment the apparent disruption in wake/sleep cycles, and the complexity of the task for the animal leading to low engagement (Koskinen et al., 2000a, 2000b), it was decided to move our attention to another paradigm involving the monitoring of arousal.

Previous studies made with the use of psychedelics relied on a low dose, with for instance 0.01 to 0.1 mg/kg of LSD, with reports of lack of engagement with the task (Carli & Samanin, 1992). As studies suggest that a medium dose of 5-MeO-DMT as the one used in this project can compare to higher doses of psilocybin in human (Barsuglia et al., 2018), the risk of non-engagement with the task or reward after an injection of 5-MeO-DMT was high. Training the animals to perform the 5-CSRT task is a long and time-consuming endeavour. We determined that the elevated risks of poor engagement and performance after such a training phase potentially outweighed the benefits. The analysis of the EEG data showing sleep-like EEG activity patterns during wakefulness in the presence of the drug were obtained at that time, and therefore the prospect of analysing the relationship between this cortical activity and arousal levels was deemed more scientifically tractable.

#### **4.4.2 A novel approach to oculometry and behaviour monitoring**

One of the reliable -albeit indirect- ways to measure arousal in rodents is to monitor pupil dilation. Indeed, studies on neuronal activity of the LC suggest direct or indirect projections to the Edinger–Westphal nucleus, responsible for the cholinergic-mediated dilation of the pupil (Joshi & Gold, 2020; R. S. Larsen & Waters, 2018; Reimer et al., 2016). Being directly correlated with NA neurotransmission through the activation of NA- $\alpha$ 2 receptors, pupil mydriasis in mammals can indicate high levels of arousal and is associated with high levels of focus and engagement (Carro-Domínguez et al., 2023; Cazettes et al., 2021; Rattenborg & Ungurean, 2023; Yüzgeç et al., 2018).

In this chapter, an adaptation of the device used in birds to measure pupil diameter in a freely moving mice was presented. This new oculometre not only allows measurement of pupil dilation, but pilot observations also suggest it can be used to monitor any interaction the animal has with its environment from the point of view of the animal. Such observations already showed that mice are not primarily relying on their visual system for exploration, as the pupils were most of the time located in the posterior part of the eye and not oriented towards the object of exploration. Indeed, it has previously been showed that should mice rely on their visual system to hunt, they would prefer to turn their heads rather than move their eyes toward the target (Michaël et al., 2020).

The oculometre was designed to minimise stress and to allow the expression of spontaneous behaviour by the animal. It can also be argued that a stressful environment can strongly alter the effects of an injection of psychedelics as human reports suggest that the mindset of the participant and the environment of the study strongly impacts the human psychedelic experience (Carhart-Harris et al., 2018b). Here, the animals were left undisturbed for the entire duration of the video recordings. Our results suggest that the oculometre is an affordable, wearable device perfectly capable of measuring eye movement similar to techniques on head-fixed

animals (Cazettes et al., 2021; Yüzgeç et al., 2018) with the benefit of allowing measurement of spontaneous behaviours. The light weight of the device allowed a rapid habituation by the animals who showed nesting and grooming behaviour which did not move the camera and allowed for between 1 to 2 hours of recordings.

Recordings were nevertheless limited in time for several reasons. First, despite early recordings showing that the device would not create short term discomfort in term of heat by its proximity to the eye of the animal, the effects of long-term activation of the LED and the camera were not measured, and so were a potential risk to the wellbeing of the animals. Second, image processing and analysis from video files is a resource intensive endeavour- by recording 1 hour over 5 days, the total size of the video recordings is about 1 TB, compared to 200 GB for continuous EEG recordings for 24 hours over 2 weeks. The analysis of such data requires an elevated computing power accessible to today's research, relying on high-end graphical processing units and computing processing units, but expensive. This chapter was conducted on a brand-new machines with state-of-the-art processing units costing several thousands of pounds, and the data training and analysis of one batch took approximately a week. The analysis of a bigger dataset would therefore be theoretically possible, but highly expensive in term of dedicated time and resources.

To optimise the analysis time, because the effects of 5-MeO-DMT had largely dissipated within 40 minutes, it was decided to focus on the altered state itself rather than continuous monitoring of vigilance states and the recordings were stopped after one hour per session per animal. Within the hour of recording, the behaviours of the animal while wearing the device were rich and not interrupted by humans. This led to the observation that, in contrast to a head-fixed experiment, freely moving mice sleep with their eyes closed, thus compromising access to pupil data. The main interest of having a 24-hour recording of the eye would be to monitor the pupil throughout vigilance states, the lack of access to the pupil during sleep shows that an updated version of the oculometre would be needed before performing longer recordings. Such improvement could be reached by a better LED positioning to try and access pupil data through the eyelid, probably with infrared lighting. However,

data management and processing should also be addressed, in order to perform continuous recordings.

### 4.4.3 The implications of pupil mydriasis

Our data suggest that 5-MeO-DMT transiently increases arousal, as indicated by the pupil mydriasis. Surprisingly, this is concomitant to the 5-MeO-DMT induced increase of delta activity in the frontal cortex. We also report that the suppression of the theta rhythm and increase of occipital delta activity, despite following the same time course as frontal delta activity in Chapter 3, lasted for a much longer time in this current experiment. These results suggest that while 5-MeO-DMT was shown to increase delta activity and suppress theta activity, a change which might be induced by the presence of the oculometre, also suppressed the increased of frontal delta. By looking at the frontal delta activity, however, it is possible to notice a small, non-significant trend to remain above 100% from 20 to 40 minutes following the injection of 5-MeO-DMT, possibly a remnant of longer-lasting delta increase. These results points toward the existence of a frontal, delta-suppressing mechanisms sensitive to environmental changes.

Furthermore, the pupil dilation data correlated with frontal but not with occipital delta activity. These different relationships might imply the existence of two separate pathways which can exert control over the altered state induced by 5-MeO-DMT. First, one implicated with frontal delta activity and decreased cortical arousal, as well as a pupil mydriasis which, paradoxically would suggest an elevated arousal (Carro-Domínguez et al., 2023; R. S. Larsen & Waters, 2018; Yüzgeç et al., 2018). The second pathway might target the occipital delta and theta rhythms. While an elevated theta activity is usually associated with increased arousal during exploratory behaviours for instance, having a suppression of the theta activity concomitant to increased pupil diameter suggesting an elevated level of arousal is also puzzling.

Pupil mydriasis is thought to be mediated by the activation of the Noradrenergic- $\alpha 2$  receptor, *via* increased noradrenergic release from the LC associated with increased of arousal levels which will lead to pupil dilation (R. S. Larsen & Waters, 2018). Observing pupil mydriasis therefore suggests that 5-MeO-DMT's action on the brain is not confined to the 5-HT system but might also have knock-on effects on the noradrenergic system. The locus coeruleus is directly impacted in the regulation of arousal, with microarousals during sleep being linked to increases in NA levels and LC activity (Osorio-Forero et al., 2021). Novel studies on human pupil diameter also report that levels of arousal during SWS oscillates following an infra-slow rhythm of around 0.1 Hz and that these fluctuations were mediated by the noradrenergic route (Osorio-Forero et al., 2021).

The prospect that 5-HT modulates LC activity and noradrenaline release has already received some interest, as other pupillometry experiments coupled with direct stimulation of the DRN shows that phasic firing from the DRN induces pupil mydriasis as well (Cazettes et al., 2021). This putative activation of the LC by DRN could explain the alteration of the sleep-wake cycle observed after the injection of psychedelics (Thomas et al., 2022) as LC activity has been shown to play a part in the fluctuation of arousal levels during SWS and might induce microarousals (Osorio-Forero et al., 2021). As both the LC and DRN are key nuclei implicated in the regulation of brain states, imbalances of their finely tuned mechanisms might result in a global alteration of neuronal activity patterns. This leads to the question of the circuitry underlying the psychedelic state reported in this chapter and the previous one. More specifically, are the effects reported due to a change in the firing patterns and activity of the LC and DRN, thus making them exclusively global, or whether the effects of 5-MeO-DMT can occur at the local level?

However, 5-MeO-DMT while being a non-selective agonist of 5-HT class receptors with its highest affinity being on 5-HT<sub>1A</sub>, *in vitro* studies suggest that it also has potent 5-HT reuptake inhibition properties as well as noradrenalin reuptake inhibition properties (Ermakova et al., 2022; Nagai et al., 2007). Exposition of

synaptosomes to 5-MeO-DMT did not lead to noradrenergic release, nor did it for dopamine and 5-HT, suggesting that the effects of 5-MeO-DMT do not directly influence noradrenaline-releasing nuclei such as the LC but rather lead to an increase of noradrenergic levels through blocking its reuptake.

Because during paradoxical sleep, levels of NA and activity of the LC are at their lowest point, an increased noradrenergic level could be responsible for PS delay reported in Chapter 3 (Crochet & Sakai, 1999). However, the time course of pupil dilation seems to suggest that arousal levels return to baseline levels at a similar time as EEG slow wave activity does, and paradoxical sleep is delayed much longer.

It seems the increase of pupil diameter can be mediated by increased noradrenaline, and suggests an increased arousal, but it is concomitant to increased EEG slow wave activity with neuronal off periods and reduced EEG theta activity which are typically associated with sleep and low arousal. This raises more questions and might suggest that slow waves are not always associated with a decrease in arousal.

The observation of slow waves in mice during wakefulness and during particularly active behaviour such as high-speed running in a wheel support this idea (Fisher et al., 2016). With these results, it is therefore possible to infer that while states of low arousal such as SWS facilitate slow wave generation, slow wave's underlying mechanisms are not entirely dependent on arousal levels.

#### **4.4.4 Conclusion**

In this chapter, two aspects of wakefulness were investigated: attention and arousal. These two processes are known to be modulated during vigilance states through monoaminergic neurotransmission. There are many procedures to monitor attention in rodents. However, the results presented here with the 5-CSRT task failed to reproduce the observations that sleep deprivation alters attentional processes, and subsequent analysis of 5-MeO-DMT effects on attention were not undertaken in favour of monitoring arousal.

To this end, the wearable oculometre was successfully built and tested to record pupil dilation, a proxy of arousal, in freely behaving rodents. Results from this new device suggest a marked increase of arousal due to the injection of 5-MeO-DMT following a time course similar to the increase of EEG delta power, but not theta power. Taken together, these results suggest that 5-MeO-DMT effects are unsurprisingly manifold and might not result from the activation of the same target. While pupil dilation and indirectly increased arousal might paradoxically be associated with sleep-like slow wave activity in the cortex, akin to those observed in mice running in a wheel, the suppression of the theta rhythm might be dependent on other factors.



# 5

## The effects of 5-MeO-DMT on auditory evoked response potentials

### Contents

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|            |                                                                                      |            |
|------------|--------------------------------------------------------------------------------------|------------|
| <b>5.1</b> | <b>Introduction</b>                                                                  | <b>128</b> |
| 5.1.1      | The auditory system and perception of auditory stimulation                           | 128        |
| 5.1.2      | The alteration of evoked response potentials                                         | 131        |
| 5.1.3      | Experimental aims                                                                    | 132        |
| <b>5.2</b> | <b>Methods</b>                                                                       | <b>133</b> |
| 5.2.1      | Animal husbandry                                                                     | 133        |
| 5.2.2      | Surgery                                                                              | 135        |
| 5.2.3      | Injections                                                                           | 135        |
| 5.2.4      | Auditory stimulations                                                                | 136        |
| 5.2.5      | Analysis                                                                             | 137        |
| <b>5.3</b> | <b>Results</b>                                                                       | <b>138</b> |
| 5.3.1      | EEG ERPs profile changes with vigilance states                                       | 138        |
| 5.3.2      | Auditory stimulations do not prevent the 5-MeO-DMT-induced changes of brain activity | 141        |
| 5.3.3      | 5-MeO-DMT alters the EEG auditory ERPs                                               | 143        |
| 5.3.4      | Intracortical ERP alterations through vigilance states and 5-MeO-DMT injections      | 145        |
| <b>5.4</b> | <b>Discussion</b>                                                                    | <b>148</b> |
| 5.4.1      | Evoked responses during sleep and wake                                               | 148        |
| 5.4.2      | The environment-dependent effects of 5-MeO-DMT                                       | 150        |
| 5.4.3      | 5-MeO-DMT induces higher-order changes                                               | 151        |
| 5.4.4      | Conclusion                                                                           | 153        |

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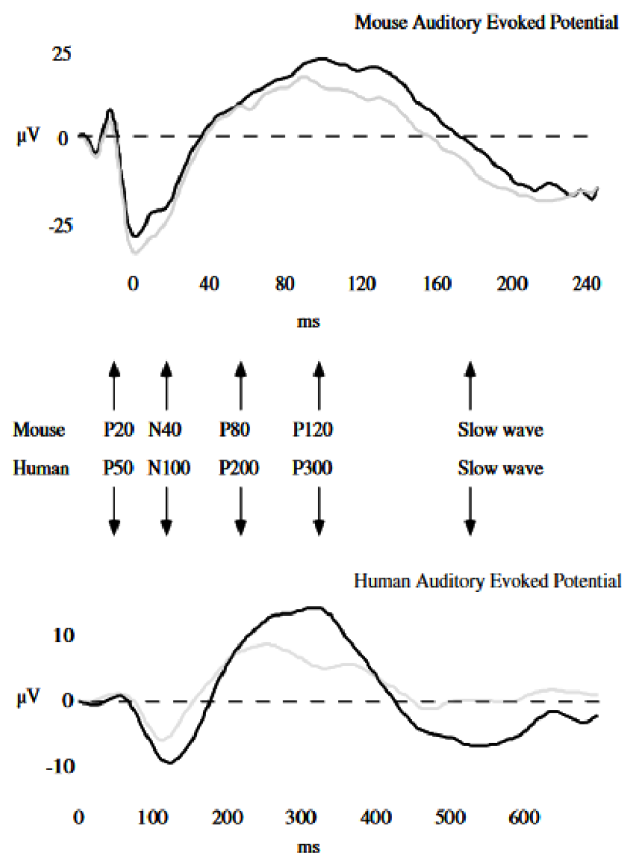
## 5.1 Introduction

### 5.1.1 The auditory system and perception of auditory stimulation

Behaviour and experiences are often driven by awareness of sensory perception which is largely influenced by sleep and wake states. Sleep is a state of low arousal and is characterised by a reduction of behavioural responses to external stimuli (Campbell & Tobler, 1984). This reduction is thought to be due to the hyperpolarisation of thalamic neurons. This thalamic gating prevents most peripheral stimuli from being relayed to the cortex (McCormick & Bal, 1994; Steriade, 2003). However, it has also been demonstrated in humans that external stimuli are integrated by the brain during sleep (Arzi et al., 2012; Blume et al., 2017; Kouider et al., 2014). Indeed, studies show that during sleep participants can differentiate between pleasant and unpleasant odours (Arzi et al., 2012), and mention of the participant's name will elicit weaker slow wave activity than spoken words with less meaning (Blume et al., 2017). It has also been shown that even through sleep, task-specific electrophysiological responses to stimuli acquired during wake are preserved (Kouider et al., 2014).

Experiments on meaning-associated stimulations in rodents also suggest that higher-order signal processing still occurs during sleep, as auditory stimuli that have been conditioned to aversive outcomes resulted in a higher disruption of SWS and PS EEG (van Kronenberg et al., 2022). Because of the immobility and low level of behavioural arousal occurring with sleep onset, sleep is inherently putting the sleeper in a more vulnerable position to predation or other external threats (Cirelli & Tononi, 2008; J. M. Siegel, 2005).

The presence of a neurophysiological mechanism, such as thalamic gating, might prevent the arousal from meaningless or non-threatening stimuli while allowing more important stimuli to be processed. This would, therefore, seem a useful process protecting the integrity of sleep and the potential survival of the sleeper.



**Figure 5.1: Comparison of evoked responses between mice and humans.** From S. J. Siegel et al. (2003): “(top) Mouse ERP (recorded in the hippocampus) to novel (black) and standard (gray) across allstrains and drug treatment conditions. (bottom) Human ERP responses (EEG, location unspecified) to novel (black) and standard (gray). Note that the human P300 and mouse P120 display increased amplitude following novel stimuli. [...] the time scale for mice is 40% that in humans and the amplitude of evoked potentials is greater in mice due to the use of intracranial electrodes as compared to scalp electrodes in humans.”

The pathways to process information coming from different kinds of external stimulation are broadly similar. The stimulus responses are first acquired by a sensory organ, then transmitted to the thalamus for further processing, and then to the cortex. It is no different for auditory stimulations: first, the signal is acquired and transformed into an electric signal by the cochlea which then projects towards the brainstem and the inferior colliculi for stimulus analysis and complex pattern recognition. The medial geniculate body of the thalamus then receives the output from the colliculi and relays the signal by projecting to the cerebral

cortex (Pickles, 2015). Evidence from recordings in cats shows that the primary auditory cortex is organised tonotopically and responds to the spatial location of the sound (Brugge et al., 1996). This pathway from the periphery to the cortex is known as a centripetal pathway. In addition, the auditory system is known to have a centrifugal component in which feedback from the cortex can affect upstream responses to the stimulation, thus allowing a finer tuning of the signal by the cortex itself (Bajo & King, 2012). This centrifugal information pathway is thought to be modulated by attentional processes.

Presentation of auditory tones in mice elicits a stereotypical evoked response potential (ERP) with phase-locked potential changes in populations of neurons. In rodents, the signal first starts with a positive deflection 20 ms after auditory stimulation and is thus labelled P20, followed by a negative deflection at 40 ms (N40), and then a larger positive deflection occurring 120 ms post-stimulation (P120) (Connolly et al., 2004; Maxwell et al., 2004; S. J. Siegel et al., 2003). These components are not specific to rodents, as similar elements of an ERP have been observed in humans with greater delay and are thought to allow cross-species comparisons of evoked responses to auditory stimulations (S. J. Siegel et al., 2003) (Figure 5.1).

The ability of the cortex to modulate the signal through the centrifugal pathway has been called sensory gating and results suggest that the amplitude of the N40 trough is dependent on sensory gating and cortical processes. Indeed, increasing doses of Olanzapine, a dopamine and 5-HT receptor antagonist, increased the amplitude of N40, hinting at a potential role of the 5-HT<sub>2A</sub> receptor in cortical sensory gating (Maxwell et al., 2004). The P120 component, on the other hand, has been linked to novelty detection processes, with its amplitude increasing in the presence of a novel tone, thus further supporting the idea that each component of an ERP can reflect a different aspect of sensory processing (Connolly et al., 2004; S. J. Siegel et al., 2003).

Despite the thalamic gating during sleep, tones played across different vigilance states resulted in cortical ERPs across all states, suggesting perception of the

stimulation (Atienza et al., 2001; Nir et al., 2015). It was also found that during sleep, while ERP components were still present, the overall amplitude of the ERP was greatly increased (Weitzman & Kremen, 1965). With the notion that the amplitude of a response is correlated with the attention paid to that stimulus, then the question of why the amplitude of the ERP is bigger during a state of low arousal remains.

### **5.1.2 The alteration of evoked response potentials**

Studying ERPs and components of auditory ERPs can provide an insight into higher cognitive processes and information processing, as well as how certain contexts or states can alter them. They can also provide a better understanding of the mechanisms underlying the alteration of perception as the one reported after psychedelic intoxication in humans. It has been reported that stimulations made during auditory hallucinatory episodes in humans led to EEG ERPs with increased latency and lower amplitude in the human N100 component, equivalent to the N40 in rodents (Hubl et al., 2007; Pinheiro et al., 2017; van Lutterveld et al., 2011). In humans, the P300 component occurring 300 ms post-stimulus and equivalent to the P120 in rodents, has been shown to have a lower amplitude in sleep-deprived patients during attention-driven tasks (Muller-Gass & Campbell, 2019). This P300 component has also been shown to be significantly reduced during psilocybin intoxication (Bravermanová et al., 2018). As both sleep deprivation and psychedelic intoxication are known to alter processes involved with sustained attention, the alteration of later ERP component following sleep deprivation and psychedelic intoxication suggests that these components might be implicated in sustained-attention processes (Bravermanová et al., 2018; Muller-Gass & Campbell, 2019).

In rodents, ketamine has also been shown to alter later ERP components. Stimulation conducted after ketamine administration showed the P120 component replaced by a strong negative component, similar to ketamine in humans (Ehrlichman et al., 2008; S. J. Siegel et al., 2003). Therefore, the study of ERPs can be used to

as a tool to investigate the effects of mind-altering drugs.

Psychedelics are still known today mainly as hallucinogens. Such a name strongly points towards alterations in perception. The name “psychedelic” however was chosen because the profound changes in emotional experiences they elicit are stronger than their hallucinogenic components (Griffiths et al., 2006; Osmond, 1957; Vollenweider et al., 1998). Studies on evoked responses in humans in the context of psychedelic research is often associated with facial expression recognition to reflect their effect on emotional experiences. On the acute effect of psilocybin, it was shown that psilocybin decreased the overall amplitude of P300 compared to placebo, but it also produced a stimulus-dependent response, as neutral faces were associated with the lowest P300 response while emotional faces were associated with a higher one, although still lower than in the control condition (Kometer et al., 2012). Long-term users of psychedelics also show alteration of ERPs with an increased amplitude of the N200 component (Orłowski et al., 2023).

These two results suggest that while the acute effects of psychedelics might impact attentional processes as illustrated by alteration of later ERP components, they also might induce long term changes in information processing. Further studies in rodents might help better understand these two different aspects. However, the effects of psychedelics on rodent ERPs have received very little to no interest so far.

### 5.1.3 Experimental aims

The notion that sleep is an inactive or disconnected state has been challenged by the observation that external stimuli induce evoked responses of higher amplitude in the cortex, and that higher order processes still occur. The cortical processes involved in the regulation of the response induced by such stimuli however is yet to be understood and this has become particularly relevant in clinical settings related to anaesthesia, coma, and vegetative states (Fu et al., 2021; Padilla & Domina, 2016). In humans, altered states of vigilance, such as the ones induced by psychedelics, show

acute disruption of ERP components through processes thought to be mediated by 5-HT neurotransmission. However, there is a lack of understanding of the effect of psychedelics on ERPs in rodents, despite its undoubted ability to provide an insight into the alteration of signal processes by psychedelics. The general aim of this chapter is therefore to investigate the acute effects of 5-MeO-DMT on auditory ERPs. The results provided in this chapter should set the stage for further mechanistic studies on the alteration of perception by psychedelics.

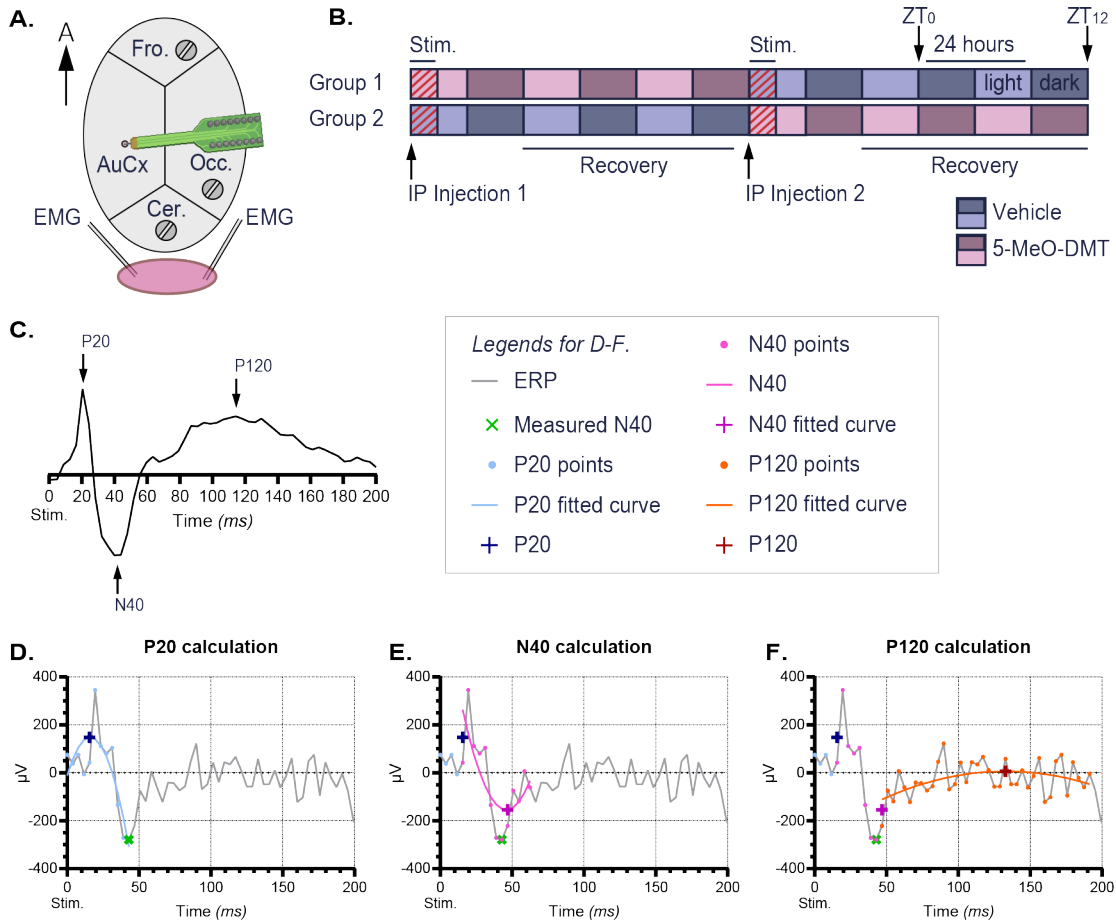
The experimental aims of this chapter are to:

1. Investigate the variations of EEG ERPs depending on vigilance states.
2. Compare EEG ERPs during wake after the injection of a vehicle solutions to the those following an injection of 5-MeO-DMT.
3. Analyse and compare ERPs in the LFP of the auditory cortex, as well as multi-unit activity, across wake, SWS, and wake with 5-MeO-DMT.

## 5.2 Methods

### 5.2.1 Animal husbandry

Adult male C57BL/6J mice ( $n = 4$ , Charles Rivers, UK, 8 to 13 weeks old), were kept singly housed in individual Plexiglas cages (20.3 cm x 32 cm x 35 cm) placed inside ventilated sound-attenuated Faraday chambers (Campden Instruments, Loughborough, UK), under a 12-12-hour light-dark cycle (10 am - 10 pm unless otherwise mentioned). The recording room was maintained at  $22 \pm 1$  °C and  $50 \pm 20\%$  humidity. Food and water were provided *ad libitum* throughout the experiment. All procedures were performed under a UK Home Office Project License and conformed to the Animals (Scientific Procedures) Act 1986.



**Figure 5.2: Experimental plans of Chapter 5.** **A.** Surgeries. Fro = Frontal. Occ = occipital. Cer = Cerebellum. AuCx = Auditory cortex. The arrow points towards the anterior part of the animal. The EMG are implanted in the nuchal muscle. **B.** Stimulation and injection protocol. The auditory stimulation protocol began immediately at light onset. Group 1 received 5-MeO-DMT first, group 2 the vehicle at light onset (ZT = 0). **C.** Averaged EEG evoked response to an auditory stimulation. The arrows point at each stereotypical components labelled P20, N40 and P120. Stim = Stimulation. **D-F.** Calculation of P20, N40 and P120 on a common signal. **D.** First is measured the minimum between 0 ms and 100 ms (Measured N40), P20 is the maximum of the 2nd order polynomial fitting the points between 0 ms and Measured N40. **E.** N40 is the minimum of the 2nd order polynomial fitting the points between P20 and Measured N40 + 20 ms. **F.** P120 is the maximum of the 2nd order polynomial fitting the points between N40 and 200 ms post-stimulation.



### 5.2.2 Surgery

All animals ( $n = 4$ ) were implanted with a 16-channel laminar probe (NeuroNexus Technologies Inc., Ann Arbor, MI, USA; model: A1x16-3mm-100-703-Z16) to perform intracortical recordings of ERPs, aiming at the auditory cortices (Figure 5.2.A). To optimise the post-hoc histological assessment of insertion depth, the tips of the electrodes were stained with the orange-red fluorescent membrane stain DiI (1,1'-Diocetadecyl-3,3',3'-Tetramethylindocarbocyanine Perchlorate; Thermo Fisher Scientific, Waltham, MA, USA) shortly before the surgery and the probes then immediately protected from light exposure to minimize bleaching. A concentration of 20 mg/ml DiI dissolved in 50/50% acetone/methanol was found to be most suitable to ensure a reliable demarcation of the electrode tract without excessive diffusion of the stain into the surrounding tissue. The laminar probes were implanted after fixation of the EEG screws and before the insertion of the EMG wires into the nuchal muscles. The targeted area was the primary auditory cortex, and the electrode was inserted at the coordinates AP = -2.46 mm, ML = +2.5 mm, DV = -2.5 mm from brain surface with a 30° angle (Figure 5.2.A). Histology showed that all electrodes were located in the parietal cortex and not deep enough to reach the auditory cortices (Figure C.1). Unfortunately, one animal was found to have pre-existing condition and was discarded from the experiment. All analyses were conducted on  $n = 3$  animals.

### 5.2.3 Injections

To monitor the effects of 5-MeO-DMT on evoked responses to auditory stimuli, the mice ( $n = 4$ ) underwent a pseudorandom and counterbalanced crossover design in which they received an injection of either vehicle (5 mL/kg saline) or 5 mg/kg 5-MeO-DMT (in 1 mg/ml saline, Beckley Psytech) on day 1, followed by two days of recovery, then the other injection condition on day 4, after which they were allowed two further days of recovery, as detailed in Chapter 2. Immediately after the injection, the auditory stimulation began (Figure 5.2.B). These animals were

also used for subsequent experiments which will be discussed in Chapter 6.

#### 5.2.4 Auditory stimulations

Open field auditory stimulations were performed in all animals. Pure tones were played through magnetic speakers (MF1 Multi-Field Magnetic Speakers, Tucker-Davis Technologies) mounted on the chamber ceilings. Tones lasted 1 second and were played every 10 seconds over 6 hours, resulting in 2160 tones being played per recording day. To reduce habituation to the tones occurring, four different frequencies were used (2 kHz, 6 kHz, 9 kHz, 11 kHz) that ascended across the optimal auditory range for C57BL/6J mice (Ison & Allen, 2003); tones were presented in a pseudorandom order which was generated by the Synapse recording software. Approximately equal numbers of each frequency were presented across the 6-hour recording. Pilot experiments in our lab had found that a sound intensity of 80 dB is optimal for open field auditory stimulation. As such, a sound level meter was used to calibrate sound intensity to this level.

Auditory evoked responses were analysed using filtered and scored EEG data, which was extracted in the window from 250 ms before stimulation, up to 125 ms after stimulation. Manual scoring of EEG epochs was used to determine the vigilance state in which each auditory stimulation occurred. The recorded ERPs showed a stereotypical shape corresponding to previous reports (Figure 5.2.C). Analysis of ERP components were conducted as follow: The start and end points of the stimulation were automatically detected through a custom MATLAB script, which also calculated the frequency of the stimulation. The electrophysiological signals were then divided into segments using the stimulation start and end points and saved with the frequency and associated vigilance state as a metadata. The analysis of the P20, N40, and P120 components was made in MATLAB (The MathWorks Inc., Natick, MA, USA) by fitting 3 second-order polynomials on the periods of interest in the ERPs (Figure 5.2.D-F). First, a theoretical N40 was detected as the

lowest trough occurring between 20 ms and 100 ms after the stimulation. The time of its occurrence labelled  $t_{40t}$ . Then, all datapoints from 0 ms to  $t_{40t}$  were used to fit a second order polynomial whose maxima would be P20, occurring at  $t_{20}$  (Figure 5.2.D). The 2nd second-order polynomial was calculated using datapoints from  $t_{20}$  to the  $t_{20} + t_{40t}$  thus having the theoretical N40 centralised. The correct N40 would be measured at the minima of the fitted curve at the time, occurring at  $t_{40}$  (Figure 5.2.E). The 3rd second-order polynomial was used to measure P120. All data points from  $t_{40}$  to 200 ms from the stimulation were used. The maxima of the fitted curve would be P120 (Figure 5.2.F). If no positive deflection was detected *in lieu* of the P20 or P120, or no negative one for N40, the analysis would immediately stop and not detect further components.

Each stimulation occurring during an epoch scored as artefactual during sleep scoring was removed from the analysis. Baseline analysis represents 6 hours of stimulations, with 1108 stimulations for animal 1, 1094 stimulations for animal 2 and 1159 stimulations for animal 3 occurring during wake : 994, 957 and 992 respectively during SWS and 61, 106 and 12 respectively during PS. Following an injection of 5-MeO-DMT, we analysed only the first artefact-free epochs of wake occurring 40 minutes following the injections, during which each animal received 241, 215 and 240 stimulations respectively during wake, or 76, 185 and 76 stimulations in the case of a vehicle injection. For the analysis of ERP components, comparisons between ERPs were performed and all animals contributed the same number of ERP, with the lowest amount of ERP available used.

### 5.2.5 Analysis

All data analysis presented in this chapter were processed in MATLAB (The MathWorks Inc., Natick, MA, USA) and analysed using MATLAB and Prism (GraphPad Software, Boston, Massachusetts USA, version 10.2.0 (392)). All data were tested for normality prior to analysis using a Shapiro-Wilk test. Non-parametric

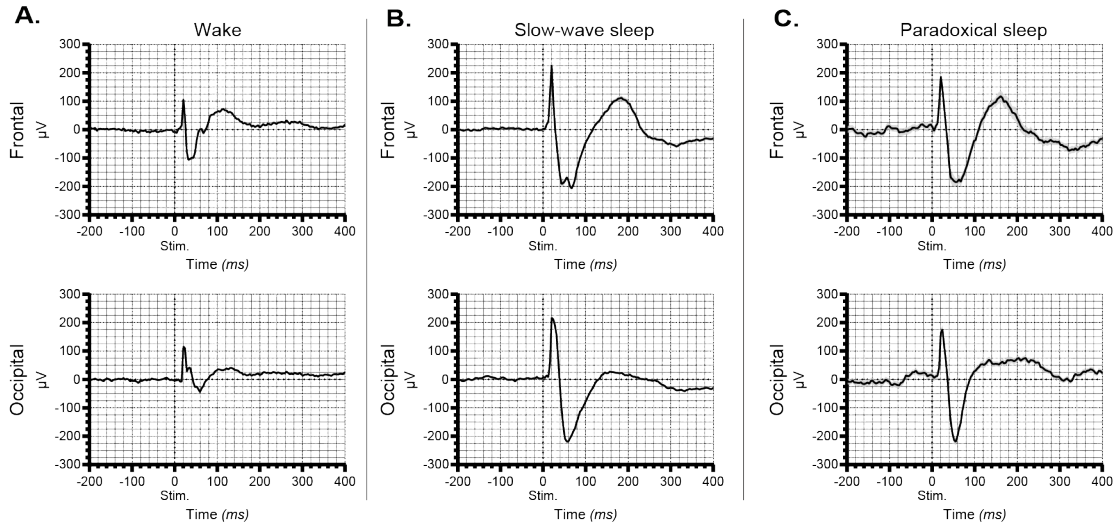
tests were used unless all conditions for a parametric test were met. To assess the difference in latency to enter a consolidated state of SWS, a paired t-test was conducted. To establish the difference in ERPs between different conditions, states, or following different stimulation frequencies, two-way ANOVAs with repeated measures together with Greenhouse-Geisser's (GG) correction were performed. The analysis of specific ERP components such as their amplitude and latency in response to different stimulation frequencies was performed with the use of a Welch ANOVA followed by Dunnett's T3 post hoc test, a regular one-way ANOVA, Brown-Forsyth ANOVA, or a Kruskal-Wallis test followed by Dunn's multiple comparisons post hoc test. The analysis of differences in specific ERP components between states or conditions was performed with a t-test or Welch's t-test. The GG correction was applied when sphericity of the data was not found. The results of the statistical analyses are detailed in Appendix C, Section C.3.

## 5.3 Results

### 5.3.1 EEG ERPs profile changes with vigilance states

Across all stimulations, auditory stimulation induced clear and stereotypical ERPs in both frontal and occipital derivations of the EEGs. In a representative animal, an initial P20 rises to around 100  $\mu$ V, followed by a N40 at around -100  $\mu$ V, and a P120 at 75  $\mu$ V (Figure 5.3.A, top). The occipital response showed a P20 reaching between 100 and 125  $\mu$ V. The equivalent of a N40 occurred at 60 ms with an amplitude of about -40  $\mu$ V, and a modest bump of 40  $\mu$ V at 130 ms can be compared to a P120 (Figure 5.3.A, bottom).

During SWS, the ERP profiles were similar to wake in some respects. In the frontal derivation, a P20 occurred and reached a height of about 225  $\mu$ V. A trough equivalent to a N40 occurred between 40 ms and 80 ms, with an amplitude between -175  $\mu$ V and -210  $\mu$ V. The equivalent to the P120 occurred much later, after 180 ms, and reached a height of about 115  $\mu$ V (Figure 5.3.B, top). While the amplitude of the SWS ERP is higher than during wake in the frontal derivation, the same is

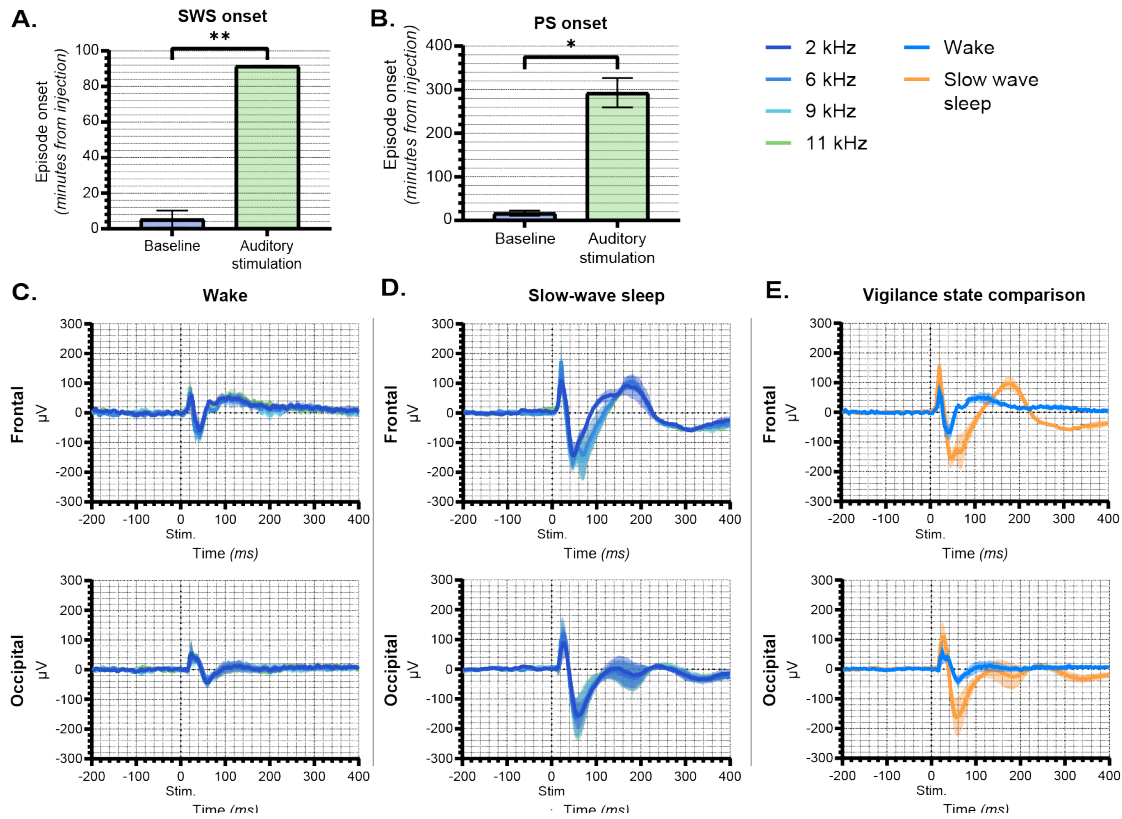


**Figure 5.3: Evoked response potential across vigilance states.** A-C. Average response in a representative animal during wake (A.;  $n = 1108$ ), SWS (B.;  $n = 994$ ), and PS (C.;  $n = 61$ ). All responses are mean + SEM of the EEG signals immediately following all stimulations of one representative animal. All ERPs have been categorised based on the vigilance state they happen and all ERPs happening during an artifact were removed. The responses between all animals were similar.

true only for P20 and N40 in the occipital derivation, with amplitudes of above 200  $\mu\text{V}$  and below -200  $\mu\text{V}$  respectively, but not for P120 with an amplitude of 25  $\mu\text{V}$ , comparable to wake, but still occurring quite late at around 150 ms after the stimulation (Figure 5.3.B bottom).

In the frontal derivation, the ERPs during PS showed a clear P20 of 180  $\mu\text{V}$ , and an N40 occurring between 40 ms and 60 ms after the stimulation, at -200  $\mu\text{V}$ . A delayed P120 occurred 150 ms after the stimulation at about 100  $\mu\text{V}$ . These results seem to suggest that the ERPs during PS have mixed features of SWS and waking (Figure 5.3.C, top). In the occipital derivation, the signals reached SWS-like amplitudes, with wake-like latencies. A P20 occurred 20 ms post-stimulation at 150  $\mu\text{V}$ , and a N40 50 ms post-stimulation reaching -225  $\mu\text{V}$ . As opposed to the occipital recordings in other states, a large P120 wave occurred reaching 75  $\mu\text{V}$  (Figure 5.3.C, bottom).

However, it was noted that the auditory stimulation induced a significant delay in consolidated SWS onset, with SWS occurring 90 minutes later in the day with



**Figure 5.4: Sleep onset and ERP profiles after stimulations in different frequencies.** **A.** SWS onset on baseline day without and with auditory stimulations. **B.** PS onset on baseline day without and with auditory stimulations. **C.** EEG ERPs during wake from auditory stimulations at 2, 6, 9 or 11 kHz. **D.** EEG ERPs during SWS from auditory stimulations at 2, 6, 9 or 11 kHz. **E.** Comparison of EEG ERPs occurring during wake and sleep in both derivations. All data are presented as mean + SEM across all stimulations (\*p < 0.05; \*\*p < 0.01). N = 3 for all panels. See Table C.1 for statistical analyses.

auditory stimulation without injection than during baseline days with no stimulation and no injections (paired t-test  $t_2 = 17.40$ ,  $p < 0.01$ ) (Figure 5.4.A), and PS just under 300 minutes later (paired t-test  $t_2 = 6.96$ ,  $p < 0.05$ ) (Figure 5.4.B). Because of this delay, all animals experienced considerably less stimulations in PS than in other states, with one animal experiencing only 12 stimulations during PS. It was decided that no further analyses would be performed during PS.

To assess whether the frequency of the stimulation elicited different ERPs, each ERP was separated based on the frequency of their corresponding stimulation and the state in which they occurred (Figure 5.4.C-D). During both wake and sleep,

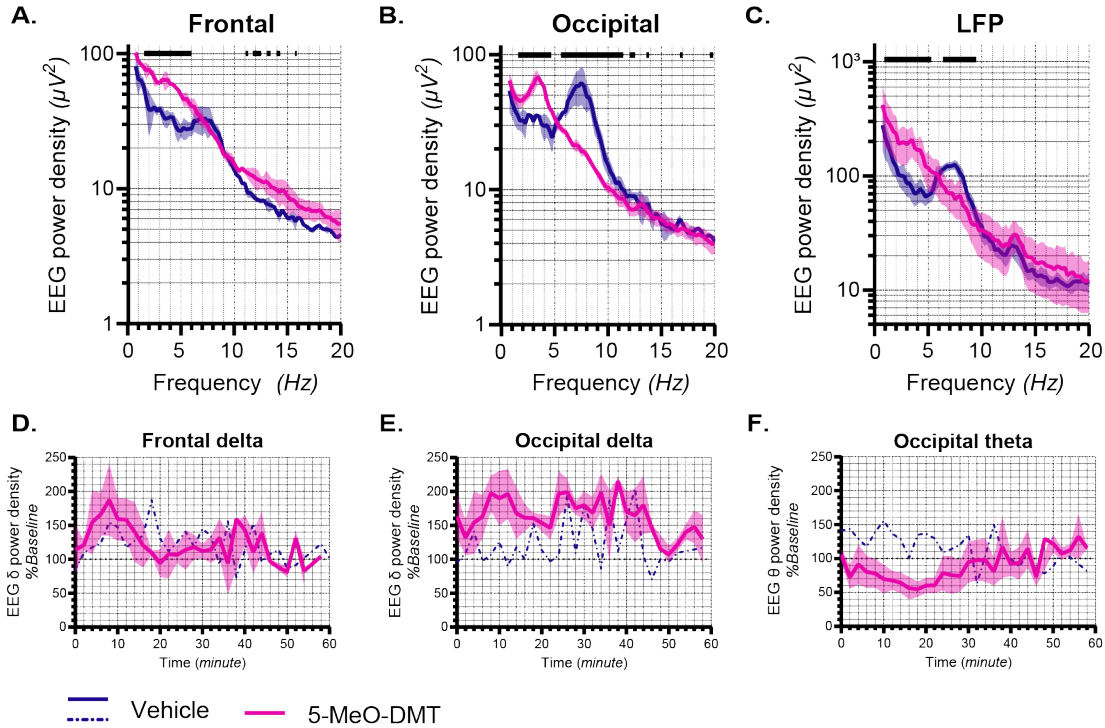
it was not possible to statistically distinguish any difference between the different stimulation rates in both frontal (RM-2-way ANOVA, wake: time point\*rate:  $F_{459,459} = 1.94$ ,  $p > 0.05$  (GG); SWS: time point\*rate:  $F_{459,459} = 0.41$ ,  $p > 0.05$  (GG)) and occipital derivations (RM-2-way ANOVA, wake: time point\*rate:  $F_{459,918} = 1.16$ ,  $p > 0.05$  (GG); SWS: time point\*rate:  $F_{459,918} = 0.50$ ,  $p > 0.05$  (GG)) (Figure 5.4.C-D). Visually, however, there is a different shape following a stimulation at 2 kHz during SWS in the frontal derivation, with a slightly sharper curve from N40 to P120 (Figure 5.4.D, top).

Because of the absence of significant difference, no distinctions between frequencies were made when comparing wake against SWS stimulations. When comparing wake ERPs to SWS ERPS, no significant difference between these two states was found in the frontal (RM-2-way ANOVA time point\*state:  $F_{459,459} = 10.83$ ,  $p > 0.05$  (GG)) and occipital derivations (RM-2-way ANOVA time point\*state:  $F_{459,918} = 1.81$ ,  $p > 0.05$  (GG)) (Figure 5.4.E). However, visual analysis suggests that stimulations during SWS produced an elongated ERP, with elevated amplitude for P20 and N40 in both derivations, and P120 in the frontal derivation reaching about 1.5 time the amplitude of wake (Figure 5.4.E). The ERP seems elongated in terms of latency during SWS (Figure 5.4.E).

To complete this analysis, the latency and amplitude each stereotypical element of all ERPs were extracted and analysed independently of their animal and were compared based on the stimulation frequency (Figure C.2-C.3) or vigilance state during which they occurred (Figure C.4-C.5). The results are detailed in Appendix C, subsections C.2.1 and C.2.2.

### **5.3.2 Auditory stimulations do not prevent the 5-MeO-DMT-induced changes of brain activity**

To make sure that the auditory stimulation did not elicit a different EEG response in the presence of 5-MeO-DMT injection, we reproduced the EEG analysis presented



**Figure 5.5: The effects of 5-MeO-DMT on EEG and LFP power spectra.** **A-B.** EEG power density in the frontal (A) and occipital (B) following an injection of 5-MeO-DMT or vehicle in the initial episodes of wakefulness occurring from 0 to 30 minutes following the injection. **C.** LFP power density in selected LFP channels following an injection of 5-MeO-DMT or vehicle in the initial episodes of wakefulness occurring from 0 to 30 minutes following the injection. **D-F.** Time course of EEG delta activity in the frontal (D) and occipital (E) derivation and theta activity in the occipital derivation (F) for 40 minutes following an injection of 5-MeO-DMT or vehicle. The dashed red line represent polynomial fitted to the delta (0 – 20 minutes) and theta (0 – 40 minutes) EEG activity. All data are presented as mean + SEM. A black horizontal line denotes a significant difference between the 5-MeO-DMT and vehicle conditions (Fisher's LSP  $p < 0.05$ ).  $N = 3$  for all panels. See Table C.2 for statistical analyses.

in Chapter 3. All raw signals showed the same characteristics associated with the psychedelic state, with sleep-like slow wave activity in the frontal and occipital derivations, as well as in the LFP derivation, including neuronal off-periods. When analysing the EEG power, the SWA was notably higher in the frontal derivation following an injection of 5-MeO-DMT compared to vehicle (RM-2-way ANOVA, frequency\*condition:  $F_{77,77} = 1.67$ ,  $p < 0.05$ ; Fisher's LSD  $p < 0.05$ ) (Figure 5.5.A), and the occipital derivation showed an increased SWA and decreased theta power compared to vehicle (ME analysis, frequency\*condition:  $F_{77,76} = 21.18$ ,  $p$

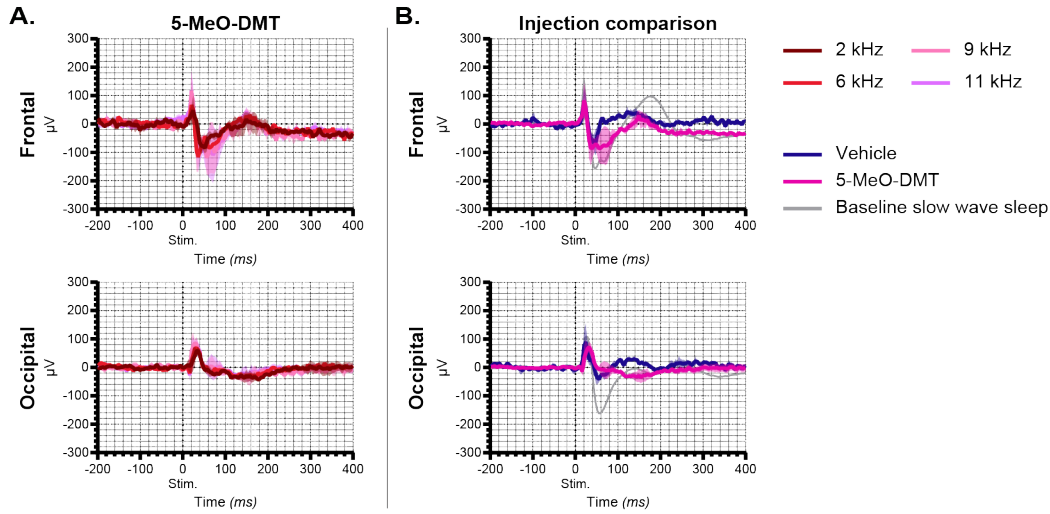


$< 0.0001$ ; Fisher's LSD  $p < 0.05$ ) (Figure 5.5.B). Regarding the LFP recordings, it was possible to observe an increase of the SWA and a suppression of the theta rhythm across all recordings (RM-2-way ANOVA, frequency\*condition:  $F_{154,308} = 10.38$   $p < 0.0001$ ; Fisher's LSD  $p < 0.05$ ) (Figure 5.5.C-E). The presence of an elevated activity rhythm around 13 Hz was also noted. This was likely artefactual due to the auditory stimulations, but further work is needed to investigate the nature of this rhythm. Removing sleep epochs from the time course of delta and theta activity data did not leave enough data to successfully estimate the activity in these two frequency bands over the course of 60 minutes in the vehicle condition (Figure 5.5.D-F). Therefore, the mean frontal and occipital EEG delta activity and occipital theta in the vehicle condition are plotted merely for display, and it was not possible to calculate the difference between 5-MEO-DMT and vehicle conditions (Figure 5.5.D-H). A good estimation of the time course of delta and theta activity however can be made. Following an injection of 5-MeO-DMT, frontal delta activity was increased by, on average, 180% relative to baseline delta activity 10 minutes following the injection and reached baseline levels 20 minutes after the injection (Figure 5.5.D). Occipital delta activity increased to 200% relative to baseline delta activity in 10 minutes, then went down to 150% at 20 minutes, and then stayed at 200% between 25 – 45 minutes following the injection before returning to baseline levels (Figure 5.5.E). Occipital theta activity slowly decreased to 50% compared to baseline theta activity in 20 minutes and then reached baseline levels 30 minutes after the injection of 5-MeO-DMT (Figure 5.5.F).

This allowed further analysis of the evoked responses during the altered state described in Chapter 3. Behaviourally, no difference was noted between the animals presented in this chapter and the mice in Chapter 3 after an injection of 5-MeO-DMT.

### 5.3.3 5-MeO-DMT alters the EEG auditory ERPs

To assess the effects of 5-MeO-DMT on ERPs, we only selected the stimuli that were occurring in the first 30 minutes following an injection of either vehicle or 5-MeO-

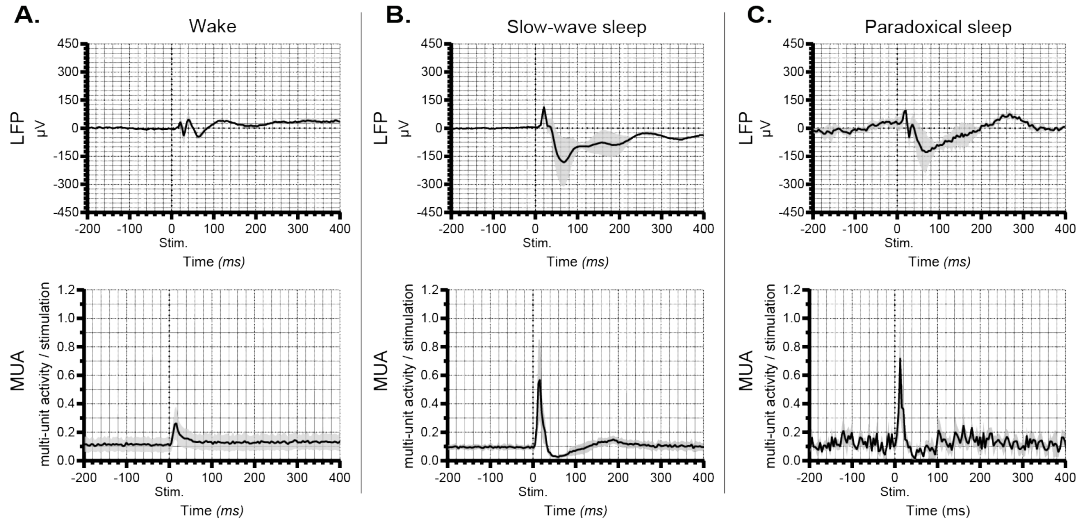


**Figure 5.6: The effects of 5-MeO-DMT on ERPs.** **A.** EEG ERPs following an injection of 5-MeO-DMT divided by stimulation frequency (2, 6, 9, 11 kHz). **B.** EEG ERPs following an injection of 5-MeO-DMT or vehicle in the frontal (top) or occipital (bottom) derivation compared with the ERPs occurring during SWS for reference. All data are presented as mean + SEM.  $N = 3$  for all panels. See Table C.3 for statistical analyses.

DMT, and during artefact-free epochs of wakefulness. All stimulation frequencies elicited the same ERP after an injection of 5-MeO-DMT in the frontal (RM-2-way ANOVA, time point\*rate:  $F_{459,459} = 1.27$ ,  $p > 0.05$  (GG)) or occipital derivation RM-2-way ANOVA, time point\*rate:  $F_{459,918} = 0.79$ ,  $p > 0.05$  (GG)) (Figure 5.6.A). Furthermore, no statistically significant difference was found between vehicle and 5-MeO-DMT conditions in the frontal (RM-2-way ANOVA, time point\*condition:  $F_{153,153} = 2.47$ ,  $p > 0.05$  (GG)) or occipital derivation (RM-2-way ANOVA, time point\*condition:  $F_{459,918} = 3.41$ ,  $p > 0.05$  (GG)) (Figure 5.6.B).

However, visual inspection of the signals suggests some mild differences in shape. Following an injection of 5-MeO-DMT in the frontal derivation, N40 seems elongated, P120 seems delayed, expressing a gentler wave more similar to SWS ERPs. In the occipital derivation, it was not possible to distinguish any clear N40 and P120 component, P120 being replaced by a wave of the opposite direction (Figure 5.6.B).

The analyses of each component of the ERP are detailed in Appendix C, subsections C.2.3.

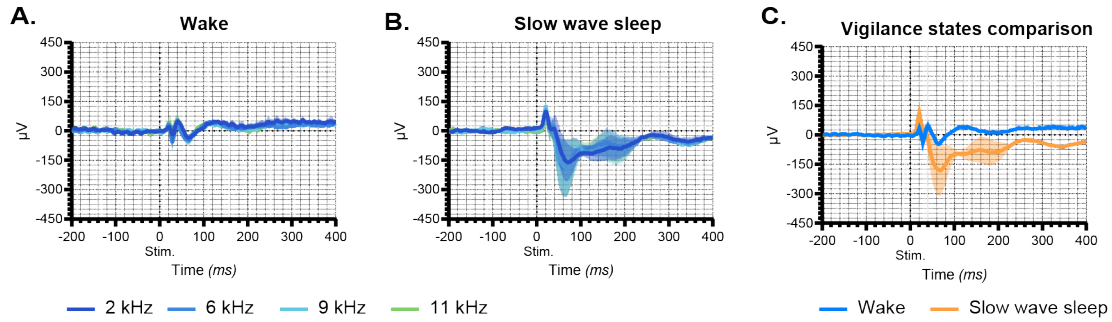


**Figure 5.7: Representative intracortical ERPs.** **A.** Average ERPs in the LFPs and corresponding multiunit activity during artefact-free episodes of wake in one representative animal **B.** Average ERP in the LFPs and corresponding multiunit activity during artefact-free episodes of SWS. **C.** Average ERP in the LFPs and corresponding multiunit activity during artefact-free episodes of PS. All data are mean + SEM of all stimulations of the best channel per animal.

#### 5.3.4 Intracortical ERP alterations through vigilance states and 5-MeO-DMT injections

To investigate intracortical ERPs, animals were implanted with 16-channel silicon probes in the parietal cortex. The location of the electrodes was confirmed with histology. All electrodes were located in the parietal cortices and were not deep enough to reach auditory areas. To compare LFP signals, one channel per animal was selected based on the signal quality and the location of the channel based on histology results. ERPs showed no proper equivalent to P20 and N40, but rather looked like a waning wave, starting with a faster frequency and rapidly decreasing in frequency, never reaching an amplitude higher than 50  $\mu\text{V}$ . The average multiunit activity (MUA) displayed a sharp increase of activity within 20 ms of the stimulation before slowly returning to a pre-stimulus level (Figure 5.7.A).

During SWS, all channels displayed ERPs of larger amplitude. A peak equivalent to a P20 was observed, reaching an amplitude of 100  $\mu\text{V}$ . A large slow wave reaching a depth below -150  $\mu\text{V}$  was observed 140 ms after the stimulation. Corresponding to these ERPs, the MUA showed a sharp rise, higher than the increase seen in

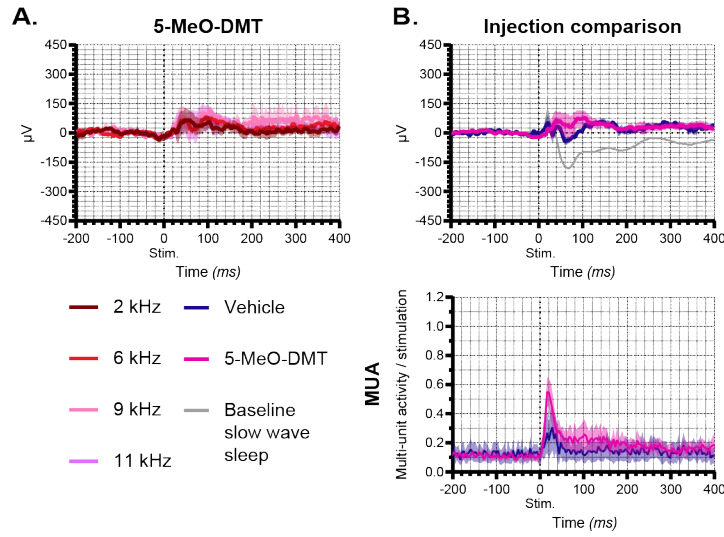


**Figure 5.8: Intracortical ERPs during wake and sleep.** **A.** ERP in the LFPs and corresponding multiunit activity during artefact-free episodes of wake divided by stimulation frequencies (2, 6, 9, 11 kHz) **B.** ERP in the LFPs and corresponding multiunit activity during artefact-free episodes of SWS divided by stimulation frequencies (2, 6, 9, 11 kHz). **C.** Comparison of LFP ERPs occurring during wake and sleep. All data are mean + SEM of all stimulations of a given frequency.  $N = 3$  for all panels. See Table C.4 for statistical analyses.

the wake state, within 20 ms of the stimulation and corresponding to the P20-like peak. After this, there was a period of longer neuronal silence from 40 ms to 80 ms corresponding to the more elongated N40-like large trough (Figure 5.7.B).

During PS, the ERP is, in a sense, more similar in shape to that seen during wake (Figure 5.7.C). While the waning shape is less obvious, the first two sharp oscillations are present, followed by a large slow wave reaching a depth of about -153 µV, 60 ms post-stimulus and with a height of 75 µV about 260 ms post-stimulus. This last positive wave is similar in shape to an EEG P120. The multiunit activity during PS, like SWS, shows a steep increase in MUA 20 ms post-stimulation, before showing a drop of activity between 30 ms and 80 ms post-stimulus, returning to pre-stimulus levels of activity from 100 ms onwards (Figure 5.7.C).

Comparing the effect of different stimulation frequencies during wake and sleep during baseline showed that there was no significant difference between all frequencies in either wake (RM-2-way ANOVA time point\*rate:  $F_{459,918} = 1.04$ ,  $p > 0.05$  (GG)) or SWS (RM-2-way ANOVA time point\*rate:  $F_{459,918} = 0.94$ ,  $p > 0.05$  (GG)) (Figure 5.8.A-B). An overall significant difference was found between wake and SWS ERPs, but no effect of time was noted (RM-2-way ANOVA, effect of state  $F_{3,6} = 93.70$ ,  $p < 0.05$  (GG)) (Figure 5.8.C). The P20 during SWS had a higher amplitude



**Figure 5.9: The effects of 5-MeO-DMT on intracortical ERPs.** **A.** ERP in the LFPs and corresponding multiunit activity following an injection of 5-MeO-DMT or vehicle during the initial period of wakefulness occurring from 0 to 30 minutes after the injection, and the average ERP during baseline slow wave sleep. **B.** ERP in the LFPs and corresponding multiunit activity following an injection of during the initial period of wakefulness occurring from 0 to 30 minutes after the injection and divided across all stimulation frequencies (2, 6, 9, 11 kHz). All data are presented as mean + SEM.  $N = 3$  for all panels. See Table C.5 for statistical analyses.

than the bi-phasic wake component, and the large negative period during SWS was not present during wake (Figure 5.8.C).

The different stimulation frequencies did not elicit any different ERPs following an injection of 5-MeO-DMT (RM-2-way ANOVA time point\*rate:  $F_{459,918} = 0.74$ ,  $p > 0.05$  (GG)) (Figure 5.9.A). Comparing the ERPs in the vehicle condition to the 5-MeO-DMT condition also did not yield any statistically significant differences (RM-2-way ANOVA time point\*condition:  $F_{459,918} = 1.04$ ,  $p > 0.05$  (GG)) (Figure 5.9.B). However, visual inspection of the signal showed little correspondence in shape between the two conditions, as an injection of vehicle elicited a response similar to that of wake, but an injection of 5-MeO-DMT induced a slow, irregular positive wave lasting 200 ms. Neither response looked like SWS ERPs. The corresponding MUA might have a higher activity following the stimulation in the 5-MeO-DMT condition compared to the vehicle condition, staying at a somewhat higher level than vehicle for about 150 ms, but no significant differences were found (RM-2-way

ANOVA time point\*condition:  $F_{459,918} = 1.45$ ,  $p > 0.05$  (GG)) (Figure 5.9.B).

## 5.4 Discussion

### 5.4.1 Evoked responses during sleep and wake

The ERPs following auditory stimulations were first assessed through the spectrum of physiological vigilance states. EEG ERPs vary strongly between wake and sleep, and less so between SWS and PS. While wake is a state known for its elevated arousal and capability to pay attention to external stimuli, the amplitude of ERPs during SWS is larger than the wake-associated ERPs, with a structure more elongated, as most components associated with ERPs are present but occur at a later time point. The origin of the larger ERP amplitude during SWS is thought to come from reduced neuronal specificity during SWS leading to the stimulus recruiting a larger population of neurons (Nir et al., 2015). The ERP profile, as mentioned, is the same across all states: ERPs in rodents are composed of an initial peak, followed quickly by a trough, before an upward slower wave. It can also be noted that during SWS, this last wave is followed by a slower negative wave in time period between 300 ms to 400 ms following the auditory stimulation.

The elongated shape of the SWS-associated ERPs has been observed in previous unpublished projects of the Vyazovski group and reported previously in humans and mice during SWS (Nir et al., 2015; Weitzman & Kremen, 1965). These results suggest that components of ERPs might be inversely proportional to the arousal state of the cortex, as the amplitudes of P20 and N40 were increased during sleep. The latency of the response might however be linked to the arousal state, as SWS was associated with delayed components. N40 has been associated with cortical gating and P120 has been correlated with sustained attention (Maxwell et al., 2004). During wake and sleep, both P120 peaks were lowered or even barely existing in the occipital derivation compared to the frontal derivation.

In the LFP recordings, state-dependent changes were also observed. Firstly, the LFP ERPs during wake are not that similar to their EEG counterpart, mainly

because of a biphasic first response leading to a waning shape of the signal. SWS and PS ERPs overall have a more elongated shape, similar to their EEG counterparts, but of much lower amplitude. SWS showed no N40 but a long, negative slow wave, while PS mixed features of both wake and SWS. The corresponding MUA to ERPs showed that MUA increases at a time equivalent to the occurrence of the P20 wave in the LFPs, in accordance with previous observations (Nir et al., 2015), and a larger negative component can be correlated with a longer period of neuronal quietness. However, in SWS and PS, no clear equivalent of P120 can be observed, with only the large slow wave occurring much later. Experiments conducted with a range of sound intensity (30 – 80 dB) shows the same results only in ERPs following high intensity stimulation and corresponding to a post-stimulus suppression of neuronal activity thought to be a physiological response to an elevated sound intensity (Nir et al., 2015).

However, this part of the chapter did not come without its limitations. First of all, during scoring, it became obvious that physiological sleep architecture was altered by the auditory stimulation. Indeed, as reported, both SWS and PS onset were significantly delayed and strong, consolidated bouts of PS were noted after the end of the stimulation period, suggesting a strong PS deprivation during the period of auditory stimulation. The PS bouts analysed in this chapter were all considered “consolidated” as they lasted more than 32 seconds. Because so few of these clean, consolidated PS episodes occurred, very few ERPs during PS were measured. PS data were presented here as representative data and not included in the remaining parts of the chapter.

The intensity of the sound was capped at 80dB in order to induce the least disturbance to the animals, based on previous experiments conducted within the group. However, the later slow wave component of ERPs measured in the LFP during SWS and PS might indeed be associated with sounds of high intensity (Nir et al., 2015), thus suggesting that the 80dB stimulus might have been too high and greatly disturbed the animals.

Then, there is a difference between the calculated components of the ERPs compared to the overall shape. First, visual comparison between the detected components and the curves shows a great accuracy in detecting the latency for P20s and N40s. However, due to the nature of the fitted curve, the amplitude is always an estimate and, more often than not, it may have underestimated the real amplitude. Visually scoring the signals in order to keep only the cleanest ERPs might correct this underestimation and result in a clearer component analysis. All comparisons between continuous ERPs were also conducted with Greenhouse-Geisser's corrections, as none of the data was homoscedastic. Homoscedasticity might be reached by a selection of clear ERPs.

### 5.4.2 The environment-dependent effects of 5-MeO-DMT

As reported in previous chapters, 5-MeO-DMT induces increased EEG slow waves in the frontal and occipital derivations, and decreased theta activity. The dynamics of these alterations, however, are not similar between one another, and even seem to differ between experiments. Indeed, in this chapter, we report a transient increase in delta activity in the frontal activity while in the occipital derivation, this increase seemed sustained over time. While it was not possible to compare the 5-MeO-DMT condition to the vehicle one, these results are similar to those observed in Chapter 4, with a sharper rise and fall of 5-MeO-DMT induced slow wave activity.

The effects of psychedelics in humans are reportedly dependent on environmental cues (Carhart-Harris et al., 2018b; Pollan, 2019), and the notion of “set and setting”, referring to the particular mindset of the participant, and the particular setting in which the psychedelic experience takes place, might indeed change the outcome of the experience itself. However, such effects have not yet been measured in rodents. More experiments are needed to test whether the frontal delta activity is sensitive to external cues and could indeed be used to determine whether the environment of the mice can influence the outcome of psychedelic intoxication.



### 5.4.3 5-MeO-DMT induces higher-order changes

After injecting 5-MeO-DMT, the ERP profiles were altered. Frontally, where in the vehicle condition all of the stereotypical components of an ERP were present, the amplitude of N40 was seemingly increased but not P120 which showed a delayed latency. In the occipital derivation, the vehicle condition again showed all the components of an ERP, whereas the 5-MeO-DMT condition replaced the N40 by a larger wave outlasting P120. Previous studies on signal components in humans and animals have linked the N40 to the centrifugal regulation of ERPs and cortical gating (Maxwell et al., 2004), thus suggesting that alteration of the N40 signal originates from the cortex and not from a pre-thalamic source. This would support the notion that psychedelic-induced alteration of perception is of a higher cortical origin rather than misperception or alteration of the pathways closer to the sensory organs.

P120 might be specifically correlated to attention-driven mechanisms (Connolly et al., 2004; Muller-Gass & Campbell, 2019; S. J. Siegel et al., 2003), even though the overall decrease of the ERP amplitude might have been linked to reduced attention towards the stimulus (Hernandez-Peon et al., 1956). A lower amplitude of the frontal P120 might illustrate the alteration of attentional processes known to result from psychedelics administration (Carli & Samanin, 1992; Koskinen et al., 2000a, 2000b; Robbins, 2002). The wider and bigger N40 might suggest a decreased cortical gating which could be interpreted as an increased capacity to recruit neurons in response to a stimulus. Such processes are thought to modulate the importance of the signal over cortical noise. However, as there is no reason to believe that other stimuli received by the animal during that time might also utilise pathways with a reduced cortical gating, one can speculate that the lack of sustained attention induced by the psychedelic and illustrated by a diminution of the frontal P120 which might be the result of a general overload of the system processing sensory information, leading to elevated background noise.

In the occipital derivation, however, the complete suppression of N40 might points towards greater cortical gating in these areas. The reported inversion of P120, not observed in the frontal derivation, support the notion that 5-MeO-DMT

impacts differently frontal cortical areas than occipital ones. While it is still poorly understood, this development can illustrate how serotonergic neurotransmission might have a finer influence on the cortex and selectively activate and inactivate certain cortical processes.

At the level of the LFP, signal alterations were also reported, notably with a large positive wave instead of the first biphasic response, followed by a waning negative then positive wave. This large positive response can be observed in the MUA as an original sharper increase, not observed on the LFP, followed by an overall increased activity. These results suggest different local responses compared to global, brain-wide ones. As the distribution of 5-HT<sub>1A</sub> receptors is known to change based on the cortical layer (Sharp & Barnes, 2020), it is possible that the differences observed in the LFP are a direct consequence of this heterogeneous distribution.

Comparing these results with dissociative drugs like ketamine also reveals a different outcome. Indeed, ketamine studies show an overall decrease in the amplitude in all components, including the P120 component (Connolly et al., 2004; S. J. Siegel et al., 2003). Ketamine is known to induce a state of disconnection which, like sleep, allows the occurrence of conscious, dream-like experiences. Being an anaesthetic, it is not surprising to observe an overall decrease of ERP amplitude, suggesting high cortical gating and low attention. Other experiments using the 5-HT<sub>2A</sub> non-selective agonist Olanzapine reported an increased N40 and pointed towards a potential role of the 5-HT<sub>2A</sub> receptor in decreasing the cortical inhibition of P20 and N40 (Maxwell et al., 2004). While Maxwell et al. (2004) also discuss other neurohormonal and neuromodulatory processes with a potential activation of dopamine D2 receptors, our results support the notion that 5-HT activation can mediate the alteration of perception commonly reported in this study. As 5-MeO-DMT is a non-selective 5-HT receptor agonist, while primarily binding to 5-HT<sub>1A</sub> receptors, further experiments are needed to differentiate the 5-HT<sub>1A</sub>-mediated effects from other 5-HT<sub>2A</sub>-mediated effects.

Overall, the ERP results presented in this chapter support the notion that 5-MeO-DMT injections alter cortical signals associated with the perception of stimuli. While the alteration of specific components suggests that the action 5-MeO-DMT might impact centrifugal, cortico-cortical pathways for perception, rather than at the primary source of the signal, further studies are required to identify the correct pathways responsible for such effects.

#### **5.4.4 Conclusion**

The experiment presented in this chapter was performed to investigate the effects of 5-MeO-DMT on EEG and LFP auditory ERPs. The results show first the changes in ERPs due to the influence of vigilance states. Notably, during SWS, a state commonly associated with environmental disconnection, the ERPs were of an amplitude larger than that in wake. The injection of 5-MeO-DMT altered wake ERPs by increasing the amplitude of P40 and reducing P210 frontally and replacing N40 and P120 by a large negative wave in the occipital derivation. While these were overall of the same magnitude as those observed during physiological wake, some occipital components like P120 were not recognisable. Moreover, LFP recordings showed channel-specific ERPs. Taken together, these results suggest that psychedelics such as 5-MeO-DMT can alter the processing underlying the perception of external stimuli. The pathway used might rely on cortico-cortical regulation as suggested by the LFP observation, with an inhibition of the cortical gating processes, leading to some ERP components of larger amplitude and changes in attentional processes. This supports the notion that the alteration of perception induced by psychedelics follows the centrifugal pathways of signal processing and does not directly alter perception.



# 6

## The potential mechanisms underlying the effects of 5-MeO-DMT

### Contents

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|            |                                                                           |            |
|------------|---------------------------------------------------------------------------|------------|
| <b>6.1</b> | <b>Introduction . . . . .</b>                                             | <b>156</b> |
| 6.1.1      | The regulation of vigilance states . . . . .                              | 156        |
| 6.1.2      | The pharmacology of psychedelics . . . . .                                | 159        |
| 6.1.3      | Experimental aims . . . . .                                               | 161        |
| <b>6.2</b> | <b>Methods . . . . .</b>                                                  | <b>163</b> |
| 6.2.1      | Animal husbandry . . . . .                                                | 163        |
| 6.2.2      | Surgery . . . . .                                                         | 163        |
| 6.2.3      | Sleep deprivation . . . . .                                               | 165        |
| 6.2.4      | Injection protocols . . . . .                                             | 166        |
| 6.2.5      | Auditory stimulation . . . . .                                            | 167        |
| 6.2.6      | Analysis . . . . .                                                        | 167        |
| <b>6.3</b> | <b>Results . . . . .</b>                                                  | <b>168</b> |
| 6.3.1      | Sleep deprivation does not alter 5-MeO-DMT-induced brain signals. . . . . | 168        |
| 6.3.2      | Infusion of 5-MeO-DMT does not affect brain activity . . . . .            | 173        |
| 6.3.3      | 5-HT <sub>1A</sub> antagonist partially restores brain activity . . . . . | 176        |
| 6.3.4      | 5-HT <sub>1A</sub> antagonist affects auditory ERPs . . . . .             | 181        |
| <b>6.4</b> | <b>Discussion . . . . .</b>                                               | <b>183</b> |
| 6.4.1      | 5-MeO-DMT-slow waves and sleep homeostasis . . . . .                      | 183        |
| 6.4.2      | 5-MeO-DMT effects might not be inducible locally . . . . .                | 186        |
| 6.4.3      | 5-HT <sub>1A</sub> and the effects of 5-MeO-DMT . . . . .                 | 188        |
| 6.4.4      | Conclusion . . . . .                                                      | 191        |

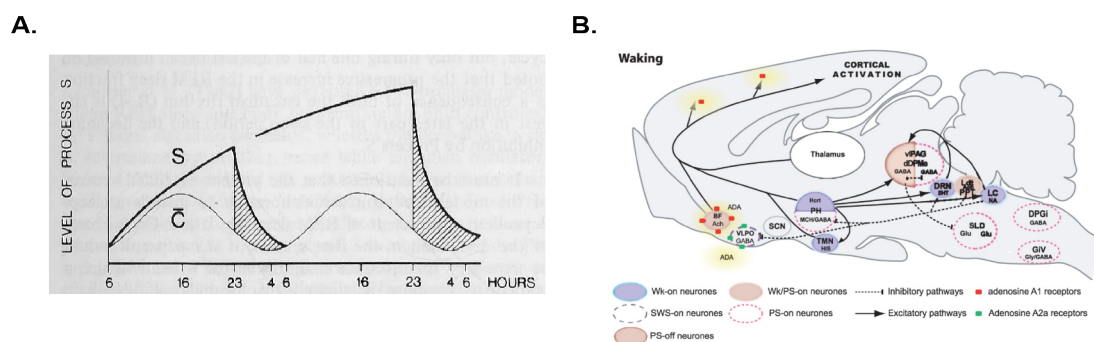
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## 6.1 Introduction

### 6.1.1 The regulation of vigilance states

Sleep regulation is traditionally described by the two-process model. This model posits that time of day and time spent in wakefulness determine the subsequent amount and intensity of sleep. To this end, the existence of two processes has been postulated: process C describes the circadian aspect, and process S the homeostatic, sleep-dependent process as described in Chapter 1, subsection 1.3.2 (Borbély, 1982; Daan et al., 1984) (Figure 6.1.A). The neuroanatomical substrates of the two processes have been investigated extensively, and several brain areas playing an important role in process C and S have been identified. Regarding process C, the suprachiasmatic nucleus of the hypothalamus has been proposed as a location of the master clock, which is regulated by retinal input and thus enables entrainment to the external light-dark cycle, and also ensures a synchronisation between different bodily systems (Moore & Eichler, 1972; Novak & Nunez, 2000; Ralph et al., 1990).

A key measurable electrophysiological marker of sleep homeostasis is the build-up of EEG slow wave activity (SWA), which increases with time spent in wakefulness and decreases with sleep (Borbély, 1982; Vyazovskiy et al., 2009a). It has been suggested that the regulation of the homeostatic process is linked to increased levels of adenosine associated with sustained wakefulness (Benington & Heller, 1995; Fort et al., 2009). Both Adenosine-1 (A1) and Adenosine-2 (A2) receptors have been implicated in sleep regulation. In humans and mice, genetic variations impacting the A2 receptor have been shown to correlate with slow wave activity and the amount of sleep time (Landolt, 2012; Urade et al., 2003). In mice, studies have shown that activation of the A1 receptor led to a dose-dependent increase of slow wave activity (Benington & Heller, 1995).



**Figure 6.1: The regulation of wake and sleep.** **A.** From Borbély (1982). Illustration of the 2-process model of sleep regulation. Process C oscillated with the circadian rhythm at 1 cycle per 24 hours. Process S increases with time spent awake and decreases exponentially with time spent asleep. **B.** From Bassetti et al. (2022). The network regulating wake and cortical activation relies on numerous nucleus and pathways “At sleep–wake transitions, the hypocretin neurones would be the first to start firing, exciting all the other waking systems (histaminergic, monoaminergic, cholinergic). In turn, these waking systems activate the thalamus and/or the cortex, leading to cortical activation and also, importantly, inhibit the GABAergic slow-wave sleep (non-rapid-eye movement [NREM] sleep)-active neurones of the VLPO and MnPn. 5HT, 5hydroxytryptamine (serotonin), Ach, acetylcholine; ADA, adenosine; BF, basal forebrain; dDPMc, deep mesencephalic reticular nucleus; DPGi, dorsal paragigantocellular reticular nucleus; DRN, dorsal raphe nucleus; GABA, gammaaminobutyric acid; GiV, ventral gigantocellular reticular nucleus; GLU, glutamate; Gly, glycine; Hcrt, hypocretin (orexin)containing neurones; His, histamine; LC, locus coeruleus; LdT, laterodorsal tegmental nucleus; MCH, melanin-concentrating hormone-containing neurones; NA, noradrenaline; PH, posterior hypothalamus; PPT, pedunculopontine tegmental nucleus; PS, paradoxical sleep; RT, reticular thalamic neurones; SCN, suprachiasmatic nucleus; SLD, sublaterodorsal nucleus; SWS, slow-wave sleep; TMN, tuberomammillary nucleus; vIPAG, ventrolateral periaqueductal grey; VLPO, ventrolateral preoptic nucleus; Wk, waking”.

Numerous studies have shown that slow waves are both a global and a local phenomenon. When slow waves have been measured throughout the cortex during slow-wave sleep, it has been shown that their intensity can vary between different cortical areas depending on the content of the prior wake experience (Kattler et al., 1994; Vyazovski et al., 2000). Furthermore, slow waves have been measured locally during physiological wake, and they have been associated with a decrease in behavioural performances and attention in both humans and rodents (Andrillon et al., 2021; Nir et al., 2017). Slow waves have also been observed in areas surrounding cortical lesions, thus further suggesting a possible involvement in recovery processes also thought to be mediated by sleep (Sarasso et al., 2020).

Most notably, the generation of slow waves occurs naturally in fundamentally disconnected networks. An injection of the anaesthetic ketamine in cats resulted in the animals exhibiting either a resting posture or a flat crouch on the floor, with no behavioural responses to external stimuli, while the cortex was producing intense bursts of slow wave activity (Miyasaka & Domino, 1968). Slow patterns of cortical activity in the delta frequency range have also been reported as a common occurrence in comatose patients (Husain, 2006). Furthermore, *in vitro* primary cultures of cortical cells of mice start to exhibit an infra slow firing pattern between 0.07 – 0.2 Hz after 10 days of in vitro culture, suggesting that a default state for cortical cultures is a slow alternation between network activity and silence (Hinard et al., 2012). Taken together, these observations support the notion that slow waves are a process originating from the cortex and for the cortex, and could correspond to a default, fundamental state. While sleep pressure is known to increase SWA and sleep acts to reduce sleep pressure, whether non-sleep-bound slow waves follow the same principles and regulatory mechanisms remains to be studied.

The circuitry underlying the regulation of vigilance states has been presented in Chapter 1.3. Pioneered by the work of the anatomists of the early and mid-twentieth century (Bremer, 1936; M. Jouvet & Michel, 1959; Magoun, 1950; von Economo, 1930), it is now established that the balance between states is mostly mediated by monoaminergic neurotransmission through the wake-promoting ascending reticular nuclei of the brain stem (Fort et al., 2009) (Figure 6.1.B). Part of these nuclei are the 5-HT releasing neurons of the dorsal raphe nucleus (DRN), whose activity correlates with wakefulness (Sakai, 2011). Imbalance of the neuromodulator systems coincides, not only with reported alteration of the sleep/wake cycle in humans with the occurrence of insomnia and hypersomnia, but it is also proposed to play a role in mental health disorders such as depression and anxiety disorders, commonly associated with 5-HT dysregulation (Geoffroy et al., 2018; Wichniak et al., 2017). Therapies for depression and anxiety targeting the 5-HT system such as MAOIs and SSRIs, in turn, also disrupt the sleep wake cycle with, for instance, a suppression of PS (Wichniak et al., 2017). It is therefore already established that pharmacological



alteration of 5-HT neurotransmission could have effects on brain activity, including oscillations which define states of vigilance.

### 6.1.2 The pharmacology of psychedelics

In recent years, it has been shown that psychedelics are a potential tool to assist psychotherapy against long-term, treatment-resistant depression (Rucker et al., 2018). As previously discussed, *classical* psychedelics are non-selective 5-HT receptor agonists, with a higher affinity for the 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> receptors (Nichols et al., 2022; O'Donnell et al., 2023). Their therapeutic properties are thought to be mediated through the activation of these receptors. Research conducted on the 5-HT<sub>2A</sub> receptor showed that these receptors were impacting hippocampal and cortical plasticity. Indeed, while the injection of DOI has been shown to increase cognitive flexibility and the rate of adaptation in the two-step reversal learning task jointly with the volume of the associated cortical area (Šabanović et al., 2024), it has also been shown to increase the expression of brain-derived neurotrophic factor (BDNF) in the cortex and to reduce the expression of BDNF in the dentate gyrus (Vaidya et al., 1997). Both of these changes in BDNF expression were prevented by an injection of a 5-HT<sub>2A</sub> antagonist prior to the injection of DOI (Vaidya et al., 1997). A recent paper also showed that other psychedelics like ketamine, LSD and psilocin had a strong binding affinity for trkB and could facilitate BDNF signalling downstream of 5-HT<sub>2A</sub> activation (Moliner et al., 2023).

This led to the speculative and purely theoretical idea that transient moments of highly elevated plasticity could be considered as a mental state on its own (Brouwer & Carhart-Harris, 2021). It could be argued that this construct does not correspond to any specific physiological mechanism, but rather tries to model acute psychological changes in humans resulting from either a traumatic and stressful event or a positive counterpart. These changes would rely, the authors speculate, on acute elevation of 5-HT levels and activation of 5-HT<sub>2A</sub> receptors (Brouwer & Carhart-Harris, 2021).

These notions correspond to the idea that psychedelics' effects on the brain happen on a global scale. As psychedelic administration in rodents is classically performed through intraperitoneal or subcutaneous routes, only the effects resulting from a global distribution of the drug in the system are reported. Few studies report the effects of direct intracortical injections to measure the local effects of psychedelics. Direct intracranial injection of DOI was sufficient to induce 5-HT<sub>2A</sub> receptor-induced HTR in rats (Willins & Meltzer, 1997) but little is known about the effects of local, intracortical injection of psychedelics on brain states and neural oscillations. We argue that such studies are important as they will not only help us to understand the effects of psychedelics on local brain networks, but also could disentangle potentially important peripheral effects of psychedelics from central effects on the behavioural and electrophysiological changes reported after their systemic administration.

The pharmacological focus of the therapeutic effects of psychedelic has been, to date, mostly concentrated on the 5-HT<sub>2A</sub> receptor. This results from the higher affinity most psychedelics have for this receptor subtype, but also because it has been shown that activation of the 5-HT<sub>2A</sub> receptors is necessary for the psychedelic experience, and HTR in rodents (Madsen et al., 2019; Vollenweider et al., 1997; Willins & Meltzer, 1997). However, among psychedelics, 5-MeO-DMT is one of the few with a higher affinity for the 5-HT<sub>1A</sub> receptor (Ermakova et al., 2022; Halberstadt et al., 2012). These receptors are densely located in the DRN and, in the layers II and VI of the cortex (Pazos et al., 1985; Riad et al., 2000). They are known to be involved in the feedback control of 5-HT neurotransmission through the inhibition of 5-HT containing neurons (Sharp et al., 1989). Given the notion that 5-HT is heavily implicated in the regulation of the sleep/wake cycle, and that impaired 5-HT biosynthesis leads to disrupted sleep onset (Sallanon et al., 1983; Tobler & Borbély, 1982), the negative feedback from activation of the 5-HT<sub>1A</sub> receptors on 5-HT neurons decreasing global 5-HT levels might be the cause of the alteration of sleep architecture and brain activity described in Chapter 3.

Previous studies reported that the increase of delta EEG activity by 5-MeO-DMT was reduced by the 5-HT<sub>1A</sub> antagonist WAY-100635 (WAY) (Riga et al., 2018), suggesting that the effects induced by 5-MeO-DMT on brain activity could indeed be mediated by the 5-HT<sub>1A</sub> receptor. However, other studies reported that the application of WAY jointly with psychedelics enhanced effects typically associated with the activation of the 5-HT<sub>2A</sub> receptors, such as HTR (Willins & Meltzer, 1997). As very little is known concerning the pharmacology underlying the effects observed in the previous chapters, the use of such an antagonist might help to identify more clearly whether the effects of 5-MeO-DMT result from a 5-HT<sub>1A</sub> or a 5-HT<sub>2A</sub> activation.

### 6.1.3 Experimental aims

The main aim of this chapter is to identify possible mechanisms responsible for the effects of 5-MeO-DMT reported in all previous chapters. We have shown that intraperitoneal injections of 5-MeO-DMT had an effect on global wake-associated brain signals, arousal, pupil dilation, and auditory ERPs. As the results suggested the occurrence of sleep-like slow waves, and because the homeostatic regulation of sleep processes induces an increased slow wave activity, we hypothesised that sleep-like slow waves observed after an injection of 5-MeO-DMT follow the same regulatory mechanisms as sleep-generated slow waves and that, therefore, sleep deprivation should increase their amplitude.

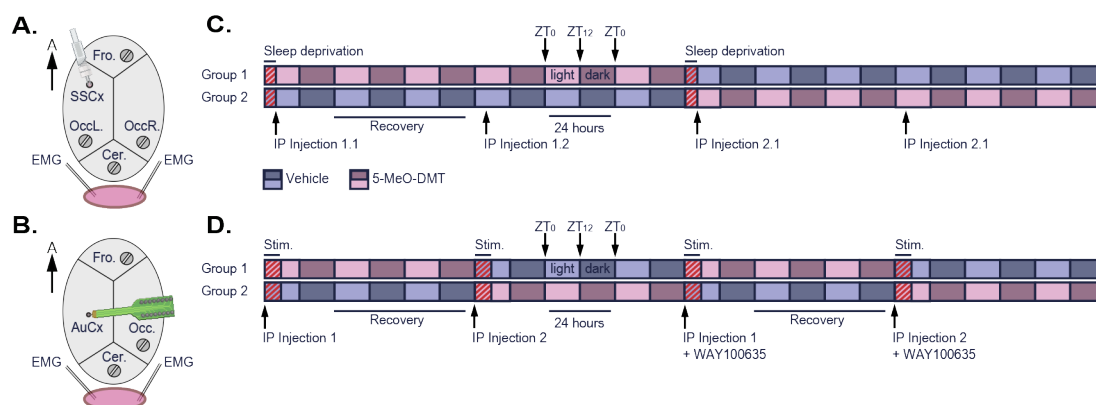
Then, as only systemic injections of 5-MeO-DMT have been conducted, the local effects of 5-MeO-DMT are not yet understood. To address this, local injections of 5-MeO-DMT into the somatosensory cortex were performed. The aim of this study was to determine whether the profound effects of the compound on brain state can be replicated at the local level, or whether psychedelics affect primarily global states of vigilance. As 5-HT<sub>1A</sub> receptors are known to promote neuronal hyperpolarisation, we hypothesised that the local injections of 5-MeO-DMT would promote the occurrence of a local state rich in slow waves comparable to the one induced in Chapter

3. Should local slow waves be observed, this would also help us understand the mechanisms underlying the generation of local and global slow waves in the cortex.

The final aim was to identify a potential receptor mediating the effects reported in the previous chapters. It was decided to use the WAY antagonist. WAY has been primarily reported to be a selective 5-HT<sub>1A</sub> receptor antagonist (Castro et al., 2008) which reportedly restores changes in behaviour induced by 5-HT<sub>1A</sub> agonists such as increased distance travelled and hole poking exploratory behaviour in the first hour following WAY administration (Halberstadt et al., 2011), as well as enhanced 5-HT<sub>2A</sub>-associated effects such as HTR (Willins & Meltzer, 1997). Some studies also suggest that WAY also activates the dopaminergic receptor D4 (Chemel et al., 2006). Binding studies in humans shows that WAY has a high occupancy rate in the DRN (Martinez et al., 2001). As 5-MeO-DMT is a non-selective agonist of 5-HT<sub>1A</sub> receptors, there is a high likelihood that the effects presented in previous chapters are mediated through the activation of many different receptor classes. The experiments presented here will assess whether some effects, such as the occurrence of a bistable state, the disappearance of the theta rhythm, the changes in arousal levels and responses to auditory stimulations, are tied to the 5-HT<sub>1A</sub> receptor.

The experimental aims of this chapter are to:

1. Assess whether 5-MeO-DMT-induced slow waves are modulated by sleep/wake history.
2. Investigate whether the alteration of local signals induced by 5-MeO-DMT requires a systemic injection.
3. Explore the role of the 5-HT<sub>1A</sub> receptor on brain activity and arousal levels.



**Figure 6.2: Experimental plans of Chapter 6.** **A-B.** Surgeries for the intracortical injections (A) and auditory stimulation (B). Fro = Frontal. Occ = occipital. Cer = Cerebellum. SSCx = Somatosensory cortex. AuCx = Auditory cortex. **C.** Sleep deprivation paradigm. The animals are sleep deprived from ZT0 to ZT4 by presenting novel object. The injections of either 5-MeO-DMT or vehicle are made at ZT4, post-sleep deprivation. Both groups are sleep deprived at the same time. Injections 1.1 and 1.2 are the same substances, injection 2.1 and 2.2 are the same substances. **D.** Stimulation and antagonist injection protocol. The auditory stimulation protocols follows the one described in Chapter 3 but is extended to allow repetition of the paradigm with the injection of WAY.

## 6.2 Methods

### 6.2.1 Animal husbandry

Adult male C57BL/6J mice ( $n = 24$ , Charles Rivers, UK, 8 to 13 weeks old), were kept singly housed in individual Plexiglas cages (20.3 cm x 32 cm x 35 cm) placed inside ventilated sound-attenuated Faraday chambers (Campden Instruments, Loughborough, UK), under a 12-12-hour light-dark cycle (10 am - 10 pm unless otherwise mentioned). The recording room was maintained at  $22 \pm 1$  °C and  $50 \pm 20\%$  humidity. Food and water were provided *ad libitum* throughout the experiment. All procedures were performed under a UK Home Office Project License and conformed to the Animals (Scientific Procedures) Act 1986.

### 6.2.2 Surgery

All animals used in this chapter underwent EEG and EMG electrode implantation as described in Chapter 2. Furthermore, three distinct subgroups of animals underwent different surgeries, with the fourth one ( $n = 8$ ) only receiving EEG and EMG

electrodes.

To investigate the effect of local administration of 5-MeO-DMT, a first group of animals ( $n = 4$ ) were implanted unilaterally with cannulae affixed with two tungsten electrodes (C315G-005T GDE/ELE 26GA/.005" TUNG 8637 RECEPTACLE, Bilaney Consultants Ltd) (4.8mm cut below pedestal; 4.7mm and 5mm electrode projection) in the primary somatosensory cortex (-1.6 mm AP, +3.3 ML, -0.5 DV from the brain surface, with an angle of 30°; Figure 6.2.A). The somatosensory cortex was selected for this experiment due to behavioural observations in Chapters 3 and 4.

Indeed, behavioural observations of Chapter 3 shows alteration in exploratory behaviour. Observations made on pupil position on Chapter 4 support the notion that mice do not rely actively on their visual system for exploratory behaviours (Michaël et al., 2020). Furthermore, observation of habituation days with oculometre showed great whiskers movement when exploring novel objects in the cages. Therefore, we hypothesised that, as mice seem to actively rely on their somatosensory system to conduct exploratory behaviours and because exploratory behaviours were altered after 5-MeO-DMT injections, the somatosensory cortex might be more strongly affected by local 5-MeO-DMT administration.

The technique followed the procedure described in subsection 2.2.3 for the implantation of 16-channel laminar probes. The implantation was performed by placing the cannula in a dummy cannula holder (Bilaney Consultants Ltd, UK) attached to the stereotaxic arm (Model 900 Small Animal Stereotaxic Instrument, David Kopf Instruments, California, USA). Upon implantation, the site was sealed with the silicone elastomer Kwik-Sil® (World Precision Instruments Inc., Sarasota, FL, USA). While not in use, during the recovery period or in the home cage of the animal when no experiment was being performed, the cannulae were covered by a dummy (C315G-005T, no projection, Bilaney Consultants Ltd). Both electrodes were located close to the opening of the cannula. The closer electrode was located +0.1 mm superior to the opening and is referred to as the “superior electrode”. The other electrode was located -0.2 mm deeper than the opening and is referred to as

the “inferior electrode”.

A second group of mice ( $n = 8$ ) was implanted with nuts in the anterior part of the skull, as well as EEG and EMG implants, to record pupil diameter and brain activity, as described in Chapter 4.

A third group of mice ( $n = 4$ ) was implanted with 16-channel laminar probes (NeuroNexus Technologies Inc., Ann Arbor, MI, USA; model: A1x16-3mm-100-703-Z16) in the auditory cortex as detailed in subsection 2.2.3, and also used in this chapter after being used previously for the experiments described in Chapter 5 (Figure 6.2.B). Unfortunately, one animal was found to have pre-existing condition and was discarded from the experiment. All analyses utilising this subgroup were conducted on  $n = 3$  animals.

Once the experiment was over, the animals implanted with intracortical electrodes or a canula ( $n = 8$ ) were perfused with a 4% PFA solution and brains were kept in phosphate buffered saline solution overnight to prepare histology slices. The brains of animals with canula implants were then stained with cresyl violet (Figure D.1) and the brains with intra-cortical, DiI-coated, silicon probes stained with DAPI (Figure C.1).

### 6.2.3 Sleep deprivation

To investigate whether slow waves induced by the injection of 5-MeO-DMT are homeostatically regulated, the mice ( $n = 8$ ) were subjected to 4-hour sleep-deprivation procedures from ZT0 to ZT4 by manually introducing novel objects into their home cages at irregular intervals (Figure 6.2.C). Novel objects included small wooden blocks, folded gloves, and various toys. This procedure is known to increase homeostatic sleep pressure and EEG slow wave activity and relies on the natural exploratory behaviour of mice (Huber et al., 2007; McKillop et al., 2018; Thomas et al., 2022). The mice were continuously monitored by the experimenters, and

novel objects were introduced into the environment as soon as the animal assumed sleep posture or as delta activity became apparent on the EEG.

### 6.2.4 Injection protocols

To investigate different regulatory mechanisms underlying the effects of 5-MeO-DMT on pupil diameter and brain activity, the mice ( $n = 26$ ) underwent a pseudorandom and counterbalanced crossover design in which they received an injection of either vehicle (5 mL/kg saline) or 5 mg/kg 5-MeO-DMT (dissolved in 1 mg/ml saline, Beckley Psytech), as described in Chapter 2 (Figure 6.2.C-D). The mice subjected to sleep deprivation ( $n = 8$ ) received their injections after sleep deprivation at ZT = 4 (1pm) (Figure 6.2.C).

The mice implanted with cannulae ( $n = 4$ ) received an intracortical injection of 5-MeO-DMT with a total volume of 500nL, at the rate of 50nL per minute. In this chapter, these intracortical injections are referred to as infusions. The cannulae were connected to Hamilton syringes (Microliter Syringe 700 series, 22 gauge 80621, Hamilton Company) with a connector cannula (0/SPR 40cm, Bilaney Consultants Ltd) and thin PE50 clear vinyl tubing (C232CT, Bilaney Consultants Ltd). The infusion rate of the Hamilton syringe was controlled by an automated pump (Harvard Apparatus Pump Controller, Harvard Apparatus), connected to the infuser (Nanomite Injector, Harvard Appara). As this method was novel, a concentration of 4.3 mg/mL was used based on previous work in rats using DOI and comparing the ED50 of DOI to the ED50 of 5-MeO-DMT in rats (Willins & Meltzer, 1997).

To investigate if 5-HT<sub>1A</sub> receptors are involved in the generation of the brain responses to systemic 5-MeO-DMT injection, an injection of 1mg/kg of WAY (634908-75-1, Sigma Merck) was performed 15 minutes before the systemic injections of 5-MeO-DMT or vehicle. The 5 mg WAY was diluted in 4 mL distilled H<sub>2</sub>O, 0.2



mL volumes were aliquoted and frozen. On the day of the injection, the solution was unfrozen and diluted in 8mL saline. The concentrations were selected based on the work by Riga et al. (2018). To control for any change induced by a double injection, the animals used for this experiment ( $n = 12$ ) first received an injection of saline followed by the 5-MeO-DMT/vehicle injection, and then the WAY treatment followed by the 5-MeO-DMT/injection (Figure 6.2.D).

### 6.2.5 Auditory stimulation

The auditory stimulation presented in this chapter was the same as presented in Chapter 5, consisting of pseudo-random presentations of tones at 2 kHz, 6 kHz, 9 kHz or 11 kHz, presented for 1 second, at a 10 second interval, for 6 hours, at 80 dB. Processing of these signals was the same as in Chapter 5. N40, P20 and P120 components were computed by fitting a second-order polynomial using MATLAB (The MathWorks Inc., Natick, MA, USA) as described in Chapter 5 (Figure 5.2.D-F). Only ERPs resulting from stimulation occurring during the artefact-free epochs of wakefulness, scored in the first 40 minutes following the injection of vehicle or 5-MeO-DMT, were included in the analysis. This amounted to 76 stimulations for animal 1, 185 for animal 2, and 75 stimulations for animal 3 following an injection of vehicle; 241, 215 and 240 stimulations following the injection of 5-MeO-DMT; 241, 147 and 189 stimulations following the injection of vehicle+WAY; and 102, 97 and 32 stimulations following the injection of 5-MeO-DMT+WAY.

### 6.2.6 Analysis

All data analyses presented in this chapter were processed in MATLAB (The MathWorks Inc., Natick, MA, USA) or DeepLabCut for the video analysis, and analysed using MATLAB and Prism (GraphPad Software, Boston, Massachusetts USA, version 10.2.0 (392)). All data were tested for normality prior to analysis using a Shapiro-Wilk test. Non-parametric tests were used unless all conditions for

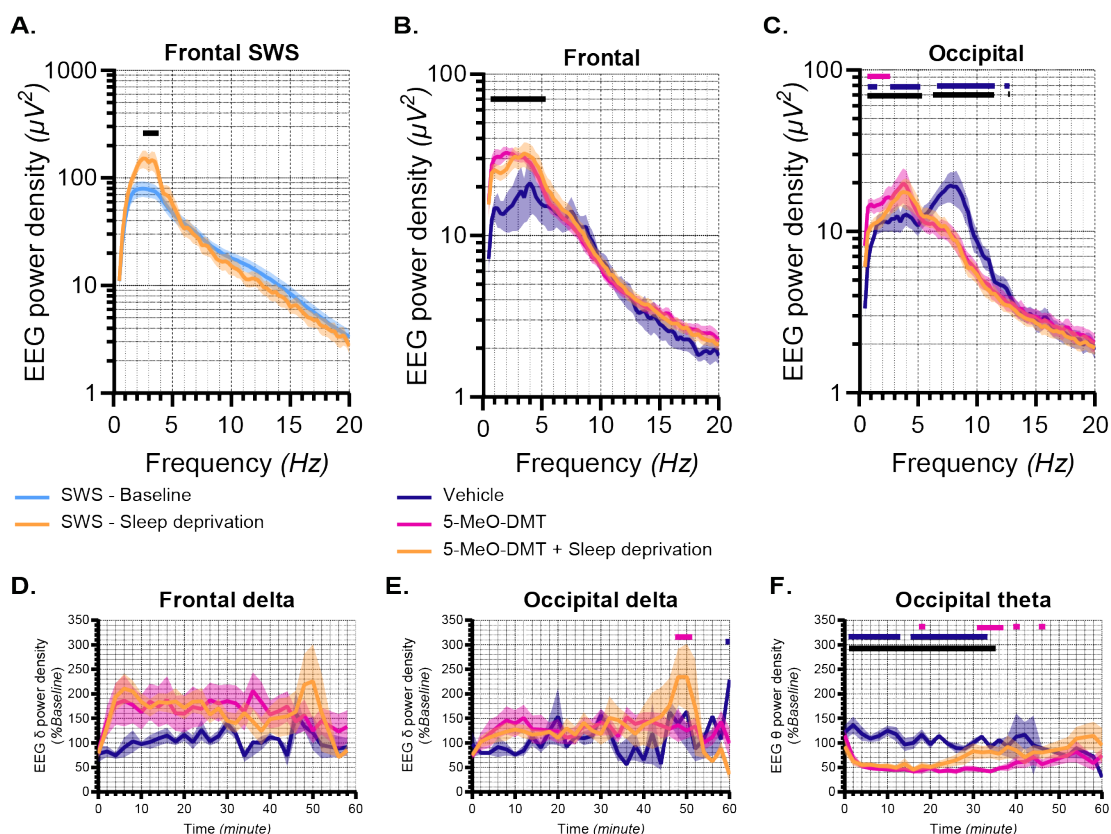
a parametric test were met. All statistics were conducted on GraphPad Prism. The analysis of EEG spectra was conducted on log-transformed values and the statistics computed with the use of a mixed-effect (ME) model and uncorrected Fisher's LSD post-hoc tests. Time course of EEG activity and pupil size were also analysed using a mixed-effects model and uncorrected Fisher's LSD, and the correlations between EEG activity and pupil size calculated using Pearson's correlation coefficients. Two-way ANOVA with repeated measures, with Greenhouse-Geisser's (GG) correction, was performed to analyse EEG and LFP ERPs. Statistics of sleep architecture and of the ERP components were computed with a one-way ANOVA followed by Tukey's post-hoc test, or a Kruskal-Wallis test followed by Dunn's test. A paired t-test was computed to assess the difference between 5-MeO-DMT and vehicle conditions on the amount of time spent asleep following an intracortical injection. The results of the statistical analyses are detailed in Appendix D, Section D.3.

## 6.3 Results

### 6.3.1 Sleep deprivation does not alter 5-MeO-DMT-induced brain signals.

To assess whether the sleep-like slow waves induced by an injection of 5-MeO-DMT were subject to sleep-homeostasis-dependent mechanisms, a cohort of animals were sleep deprived. Following a four-hour sleep deprivation protocol, SWA was significantly increased during the subsequent NREM sleep episodes, as expected from the literature (Borbély, 1982; Vyazovskiy et al., 2009a) (ME analysis, frequency\*condition:  $F_{234,1476} = 6.82$ ,  $p < 0.0001$ ; Fisher's LSD  $p < 0.05$ ) (Figure 6.3.A).

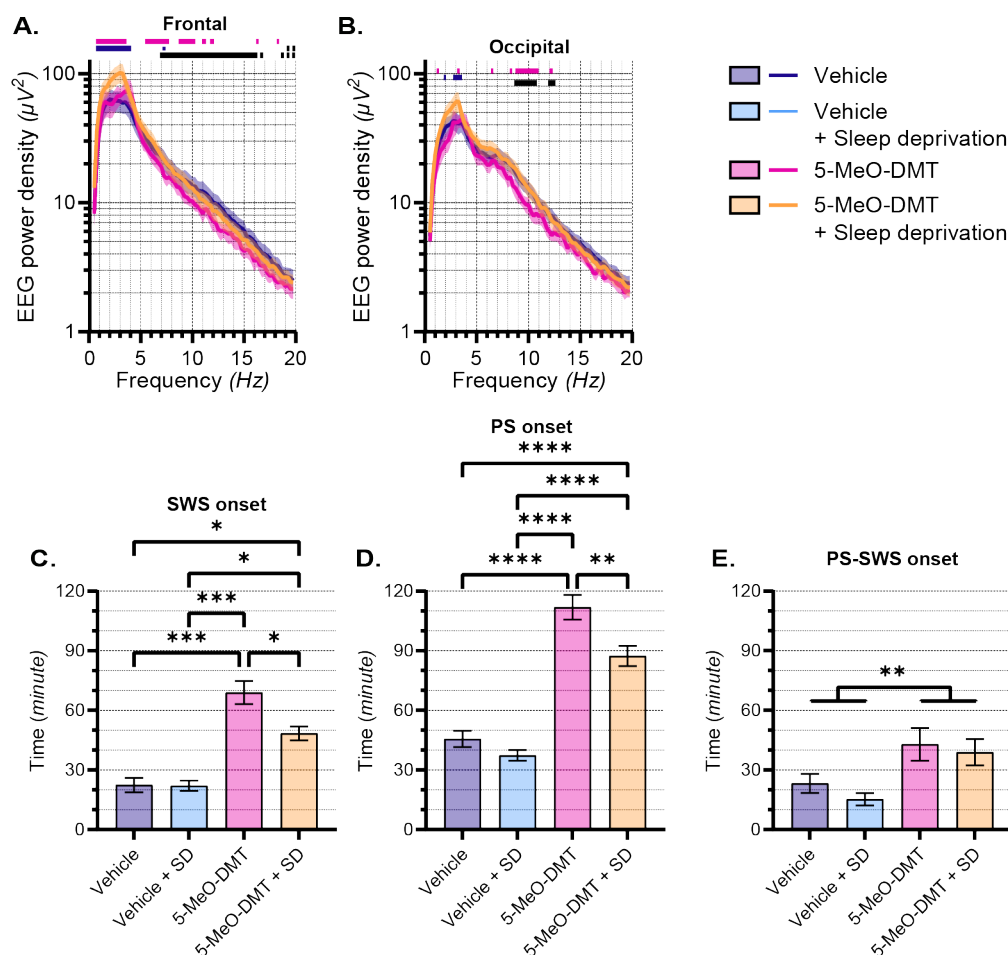
Sleep deprivation, however, did not alter the brain activity following an injection of 5-MeO-DMT during the initial 30-minute period of wakefulness post-injection. Specifically, an injection of 5-MeO-DMT, with or without sleep deprivation, increased frontal SWA (ME analysis, frequency\*condition:  $F_{156,1013} = 7.40$ ,  $p < 0.0001$ ; Fisher's LSD  $p < 0.05$ ) (Figure 6.3.B), and increased SWA and decreased theta



**Figure 6.3: The effects of sleep deprivation on 5-MeO-DMT-induced changes in EEG activity.** **A.** Effects of sleep deprivation on frontal EEG activity in the 2 hours following sleep deprivation. The black horizontal line denotes a significant difference between sleep deprivation and baseline condition (Fisher's LSD  $p < 0.05$ ). **B-C.** Effects of sleep deprivation on 5-MeO-DMT injections in the frontal (B.) and occipital (C.) EEG derivation compared to 5-MeO-DMT and vehicle without sleep deprivation. **D-F.** Time course of frontal EEG delta (D.), occipital EEG delta (E.) or occipital EEG theta (F.) activity after an injection of 5-MeO-DMT or vehicle, or 5-MeO-DMT following sleep deprivation. All data are mean + SEM. A black, pink, or blue horizontal line denotes a significant difference the 5-MeO-DMT alone conditions and the vehicle condition (black), between the 5-MeO-DMT+sleep deprivation condition and vehicle condition (dark blue), or the 5-MeO-DMT+sleep deprivation and 5-MeO-DMT alone (pink) (Fisher's LSD  $p < 0.05$ ).  $N = 8$  for all panels. See Table D.1 & Table D.2 for statistical analyses.

activity in the occipital derivation (ME analysis, effect of interaction  $F_{156,936} = 10.39$ ,  $p < 0.0001$ ; Fisher's LSD  $p < 0.05$ ) (Figure 6.3.C). However, frontal EEG spectra after 5-MeO-DMT combined with sleep deprivation were not significantly different from 5-MeO-DMT alone in the frontal derivation at any frequency bin (ME analysis, frequency\*condition:  $F_{156,1013} = 7.40$ ,  $p < 0.0001$ ; Fisher's LSD for 5-MeO-DMT and 5-MeO-DMT+SD  $p > 0.05$ ) (Figure 6.3.B). In the occipital derivation, a significant decrease was found in the 0.75 Hz to 2.5 Hz frequency range after 5-MeO-DMT injection and sleep deprivation compared to drug only, an activity comparable to vehicle-levels of activity (ME analysis, frequency\*condition:  $F_{156,936} = 10.39$ ,  $p < 0.0001$ ; Fisher's LSD for 5-MeO-DMT and 5-MeO-DMT+SD  $p < 0.05$  between 0.75 and 2.5 Hz) (Figure 6.3.C).

Next, we looked more closely at the time course of delta activity after the injection of the drug. Visual inspection of the time course of delta activity in the frontal derivation suggested an initial increase of delta activity after an injection of 5-MeO-DMT, both alone and when combined with sleep deprivation. However, no significant difference was found (ME analysis, time point\*condition:  $F_{56,217} = 0.99$ ,  $p = 0.48$ ) (Figure 6.3.D). In the occipital derivation, a significantly larger increase occurred in the 5-MeO-DMT+SD condition compared to 5-MeO-DMT alone 50 minutes after the injection (ME analysis, time point\*condition  $F_{60,210} = 1.41$ ,  $p < 0.05$ , Fisher's LSD  $p < 0.05$  5-MeO-DMT against 5-MeO-DMT+SD) (Figure 6.3.E). Concerning the occipital theta activity, we observed that 5-MeO-DMT induced a significant decrease compared to vehicle within the first 10 minutes regardless of sleep deprivation (ME analysis, time point\*condition  $F_{54,221} = 2.05$ ,  $p < 0.0001$ ; Fisher's LSD  $p < 0.05$  both 5-MeO-DMT conditions against vehicle), and differences were found between the 5-MeO-DMT alone and 5-MeO-DMT+SD condition at 20 minutes and between 30 to 40 minutes (Fisher's LSD  $p < 0.05$  5-MeO-DMT against 5-MeO-DMT+SD) (Figure 6.3.F).



**Figure 6.4: The effects of sleep deprivation on subsequent sleep episodes.** **A-B.** EEG power spectra in the frontal (top) and occipital derivation (bottom) following 5-MeO-DMT or vehicle injections. **C-D.** Time to SWS (C) or PS (D) consolidated episode onset following sleep deprivation and the injection of either vehicle or 5-MeO-DMT. **E.** Time from the onset of SWS to the onset of the first consolidated episode of PS after sleep deprivation and the injection of either 5-MeO-DMT or vehicle. A black, pink, or blue horizontal line denotes a significant difference the 5-MeO-DMT alone conditions and the vehicle condition (black), between the 5-MeO-DMT+sleep deprivation condition and vehicle condition (dark blue), or the 5-MeO-DMT+sleep deprivation and 5-MeO-DMT alone (pink) (Fisher's LSD  $p < 0.05$ ). All data are mean + SEM (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ ).  $N = 8$  for all panels. See Table D.1 & Table D.3 for statistical analyses.

Regarding the subsequent sleep episodes, in the frontal derivation we found that 5-MeO-DMT+SD increased slow wave activity compared to drug alone and vehicle (ME analysis, frequency\*condition  $F_{234,1476} = 6.82$ ,  $p < 0.0001$ ; Fisher's LSD  $p < 0.05$  5-MeO-DMT+SD against 5-MeO-DMT and against vehicle between 0.25 – 3.5 Hz) (Figure 6.4.A). However, as opposed to results presented in Chapter 3, 5-MeO-DMT alone showed a significantly lower EEG activity between 7 – 16 Hz compared to vehicle (Fisher's LSD  $p < 0.05$  5-MeO-DMT against vehicle). 5-MeO-DMT+SD has similar activity compared to vehicle from 4 Hz and above with sporadic significant difference picked up by Fisher's LSD for singular frequencies. 5-MeO-DMT alone therefore had also a lower activity compared to 5-MeO-DMT+SD between 5.5 Hz and 13 Hz (Fisher's LSD  $p < 0.05$ ) (Figure 6.4.A). In the occipital derivation, 5-MeO-DMT alone had an EEG activity lower than vehicle and 5-MeO-DMT+sleep deprivation between 9 Hz and 11 Hz (ME analysis, frequency\*condition:  $F_{156,1013} = 7.40$ ;  $p < 0.0001$ , Fisher's LSD  $p < 0.05$  5-MeO-DMT against 5-MeO-DMT+SD) (Figure 6.4.B). It was also found that sleep deprivation induced a small increase in occipital slow-wave activity during SWS with a significant difference between 5-MeO-DMT+SD when compared to vehicle or 5-MeO-DMT alone (Fisher's LSD  $p < 0.05$ ) (Figure 6.4.B).

Similar result to the ones obtained in Chapter 3 were found concerning the onset of SWS and PS. Indeed, it was found that 5-MeO-DMT injections delayed the occurrence of SWS compared to vehicle (RM-2-way ANOVA, effect of injection:  $F_{1,7} = 85.41$ ,  $p < 0.0001$ , Šídák's post hoc  $p < 0.001$ ) (Figure 6.4.C), and PS onset as compared to vehicle (RM-2-way ANOVA, effect of injection:  $F_{1,7} = 350.2$ ,  $p < 0.0001$ , Šídák's post hoc  $p < 0.0001$ ) (Figure 6.4.D).

Sleep deprivation was found to have a moderate impact on the onset of states, but especially when coupled with an injection of 5-MeO-DMT. Indeed, no significant difference was found comparing the vehicle and vehicle+SD conditions. Sleep deprivation significantly reduced NREM latency induced by the injection of 5-MeO-DMT (RM-2-way ANOVA, sleep deprivation\*injection:  $F_{1,7} = 7.11$ ,  $p <$

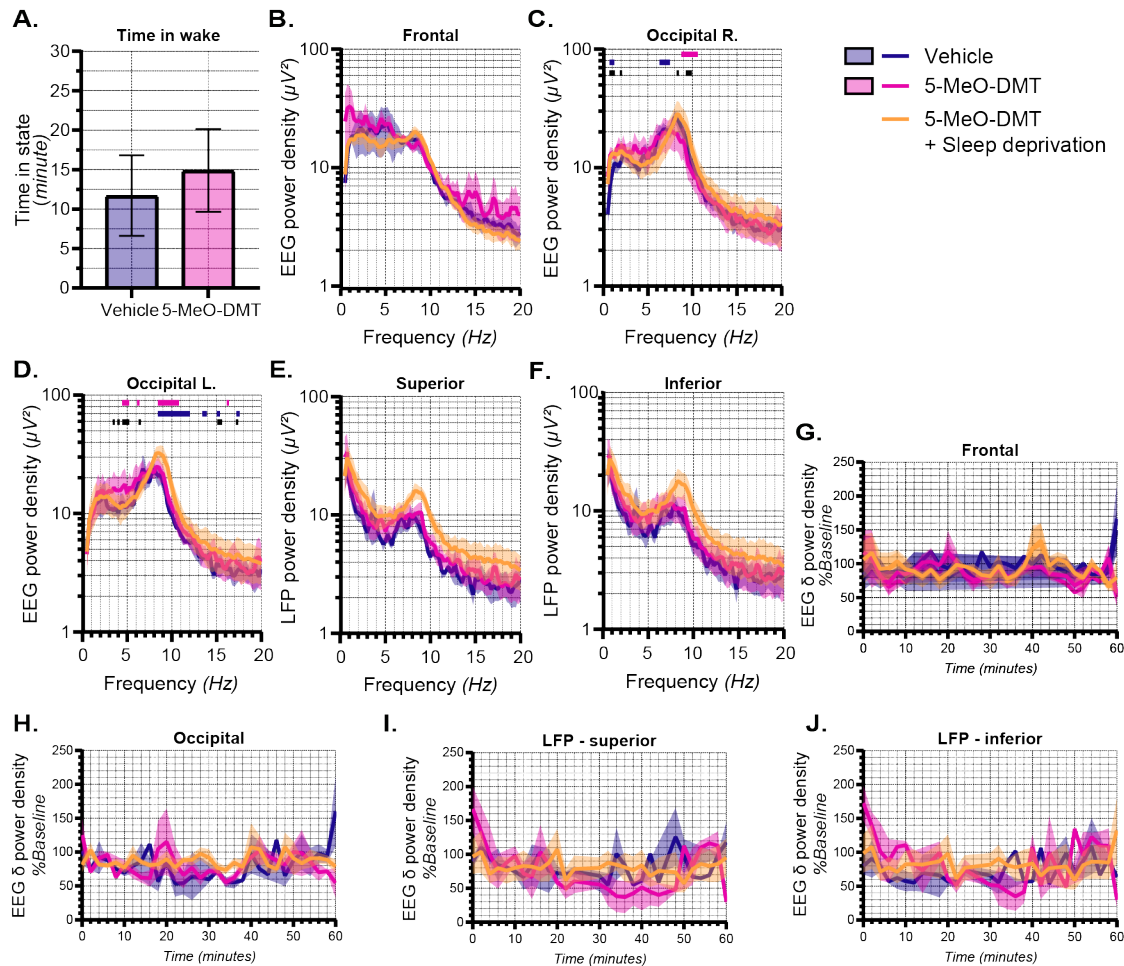
0.05; Šídák's post hoc  $p < 0.05$ ) (Figure 6.4.C), as well as PS latency (RM-2-way ANOVA, sleep deprivation\*injection:  $F_{1,7} = 11.06$ ,  $p < 0.05$ ; Šídák's post hoc  $p < 0.01$ ) (Figure 6.4.D).

The difference between PS from SWS however suggests that PS occurs after the same amount of time since SWS has elapsed for both conditions, with PS onset occurring around 40 minutes after SWS onset in both conditions and significantly later than in the vehicle condition (RM-2-way ANOVA, effect of injection:  $F_{1,7} = 25.21$ ,  $p < 0.0001$ ; effect of sleep deprivation  $F_{1,7} = 1.72$ ;  $p = 0.23$ , effect of injection\*sleep deprivation  $F_{1,7} = 0.13$ ,  $p = 0.73$ ) (Figure 6.4.E). This suggests that sleep deprivation and thus increased SWA during SWS might not alter the delay in PS observed after an injection of 5-MeO-DMT, despite PS occurring at an earlier time from the injection after sleep deprivation.

### 6.3.2 Infusion of 5-MeO-DMT does not affect brain activity

To investigate the local effects of 5-MeO-DMT, a cohort of mice were implanted with cannulae in the somatosensory cortex. Once the injectors were inserted into the cannulae, the mice rapidly showed nesting behaviour and sleep attempts, regardless of the substance infused. To make sure the effects of intracortical infusions of 5-MeO-DMT during wakefulness were measured, it was decided to perform a third infusion of 5-MeO-DMT while keeping the animals fully awake for one hour by following a sleep deprivation protocol.

It was found that from the start of the infusion until 30 minutes later, the animals spent on average,  $11.72 \pm 5.01$  minutes in wake when infused with vehicle and  $14.90 \pm 5.24$  minutes when infused with 5-MeO-DMT (Figure 6.5.A). There was no significant difference between the two conditions (paired  $t$  test  $t_3 = 0.42$ ,  $p = 0.70$ ). Thus, the sleep deprivation protocol would help to better measure changes induced to brain activity during wake by increasing the time spent in wake.



**Figure 6.5: The effects of intracortical injections of 5-MeO-DMT in the SSCx.**

**A.** Time spent in wakefulness from the start of the injection protocol in animals injected with vehicle or 5-MeO-DMT. **B-F** Effects of intracortical injections on frontal (B), occipital right (C), occipital left (D) EEG activity, and in the superior (E) or inferior (F) SSCx LFP comparing the effects of an injection of 5-MeO-DMT and vehicle, as well as 5-MeO-DMT with sleep deprivation. **G-I** Time course of delta activity in the frontal EEG derivation (G), occipital EEG derivation (H) superior LFP (I) or inferior LFP (J) activity after an intracortical injection of 5-MeO-DMT or vehicle, or 5-MeO-DMT with sleep deprivation. All data are mean + SEM. A black, pink, or blue horizontal line denotes a significant difference the 5-MeO-DMT alone conditions and the vehicle condition (black), between the 5-MeO-DMT+sleep deprivation condition and vehicle condition (dark blue), or the 5-MeO-DMT+sleep deprivation and 5-MeO-DMT alone (pink) (Fisher's LSD  $p < 0.05$ ).  $N = 4$  for all panels. See Table D.4, Table D.5 & Table D.6 for statistical analyses.



Regarding the global EEG response, the intracortical infusion of 5-MeO-DMT did not produce any consistent activity changes in any frequency band. In the frontal derivation, all curves for the different conditions were superimposed and no significant effect of condition or interaction was observed (ME analysis, frequency\*condition  $F_{156,231} = 0.93$ ,  $p = 0.68$ ) (Figure 6.5.B).

In the occipital right derivation, close to the infusion site, some minor differences were noticed. The infusion of 5-MeO-DMT resulted in minor changes compared to the vehicle condition, with a relatively small increase between 0.75 – 1.25 Hz, and similarly small decrease around 9.5 Hz (RM-2-way ANOVA, frequency\*condition:  $F_{156,466} = 1.33$ ,  $p < 0.05$ ; Fisher's LSD  $p < 0.05$  5-MeO-DMT against vehicle for specific frequencies) (Figure 6.5.C). In the 5-MeO-DMT+sleep deprivation condition, some frequencies in the theta band were suppressed compared to vehicle (Fisher's LSD  $p < 0.05$  5-MeO-DMT+SD against vehicle from 6.25 – 7.25 Hz), but then reached vehicle-like levels from 7.5 Hz onwards (Fisher's LSD  $p > 0.05$ ) to end up being significantly higher than the 5-MeO-DMT alone condition between 8.75 Hz and 10.5 Hz (Fisher's LSD  $p < 0.05$ , 5-MeO-DMT against 5-MeO-DMT+SD between 8.75 – 10.5 Hz) (Figure 6.5.C).

In the left occipital derivation, the infusion of 5-MeO-DMT alone induced sporadic significant increases of power in slow frequencies around and below 5 Hz compared to the vehicle, and compared to the 5-MeO-DMT+sleep deprivation condition (RM-2-way ANOVA, frequency\*condition:  $F_{154,462} = 1.90$ ,  $p < 0.0001$ ; Fisher's LSD  $p < 0.05$ ) (Figure 6.5.D). While activity in the theta frequency band was similar between vehicle and 5-MeO-DMT alone, 5-MeO-DMT+sleep deprivation showed an increased theta activity compared to both of these conditions (Fisher's LSD  $p < 0.05$ ) (Figure 6.5.D).

At the local level, in the somatosensory cortex, the drug infusion did not induce major changes compared to the vehicle condition (Figure 6.5.E-F). In both derivations, no interaction was found (RM-2-way ANOVA with repeated measures, superior LFP: frequency\*condition:  $F_{156,468} = 0.68$ ,  $p = 0.99$ ; inferior LFP: frequency\*condition:  $F_{156,468} = 0.67$ ,  $p = 0.99$ ). However, in the LFP of the

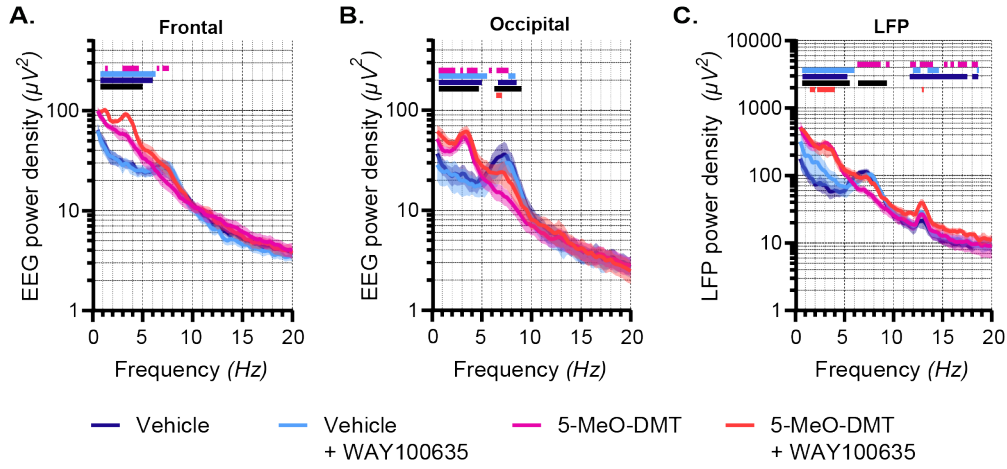
superior electrode, an effect of condition was found (effect of condition  $F_{2,6} = 6.09$ ,  $p < 0.05$ ) with a significant difference between vehicle and 5-MeO-DMT+sleep deprivation (Tukey's post hoc  $p < 0.05$  5-MeO-DMT against vehicle) (Figure 6.5.E), with a tendency for the 5-MeO-DMT+sleep deprivation condition to increase global power density (Figure 6.5.E-F).

Analysis of the dynamics of delta activity in the frontal derivation (Figure 6.5.G), LFP of the superior electrode (Figure 6.5.H) and inferior electrode (Figure 6.5.I) did not reveal any differences between conditions. In the 5-MeO-DMT alone and the vehicle conditions the animals spent a large proportion of the time during the interval we studied asleep. As only wake episodes were kept in this analysis, more sporadic data occurred the more the recording went on. No significant difference between all conditions was found in the frontal (ME analysis, time point\*condition:  $F_{58,12} = 0.96$ ,  $p = 0.58$ ) (Figure 6.5.G) and occipital derivations (ME analysis, time point\*condition:  $F_{56,59} = 1.20$ ,  $p = 0.24$ ) (Figure 6.5.H). No difference was found in the superior electrode LFP (ME analysis, time point\*condition  $F_{52,51} = 1.43$ ,  $p = 0.10$ ) (Figure 6.5.I) and inferior electrode LFP (ME analysis, time point\*condition  $F_{54,52} = 1.47$ ,  $p = 0.08$ ) (Figure 6.5.J).

### 6.3.3 5-HT<sub>1A</sub> antagonist partially restores brain activity

5-MeO-DMT is known to bind primarily with the 5-HT<sub>1A</sub> receptor. To investigate how much of the signal alteration is due to an activation of the 5-HT<sub>1A</sub> receptor, we injected WAY-100635 (WAY), a known 5-HT<sub>1A</sub> antagonist, 15 minutes prior to the systemic injections of 5-MeO-DMT.

In the frontal derivation, the injection of WAY coupled with vehicle did not result in any significant changes (Figure 6.6.A). The injection of 5-MeO-DMT alone resulted in a significant increase of frontal slow wave activity, as reported in previous chapters (ME analysis, frequency\*condition:  $F_{234,1009} = 11.49$ ,  $p < 0.0001$ , Fisher's LSD  $p < 0.05$ ) (Figure 6.6.A). Coupling 5-MeO-DMT with WAY not only resulted



**Figure 6.6: The effects of 5-MeO-DMT on EEG and LFP spectrum with a 5-HT<sub>1A</sub> receptor antagonist.** A-C. Effects of intracortical injections on frontal (A), occipital (B) EEG activity, and in the LFP (C) comparing the effects of an injection of 5-MeO-DMT and vehicle, as well as 5-MeO-DMT+WAY and vehicle+WAY 100635. All data are mean + SEM. A red, black, pink, or dark or light blue horizontal line denotes a significant difference the two vehicle conditions (red, bottom), 5-MeO-DMT alone condition and the vehicle condition (black), between the 5-MeO-DMT+WAY condition and vehicle condition (dark blue), between the 5-MeO-DMT+WAY condition and vehicle+WAY condition (light blue), and the two 5-MeO-DMT conditions (pink, top) (Fisher's LSD  $p < 0.05$ ).  $N = 9$  for all panels. See Table D.7 for statistical analyses.

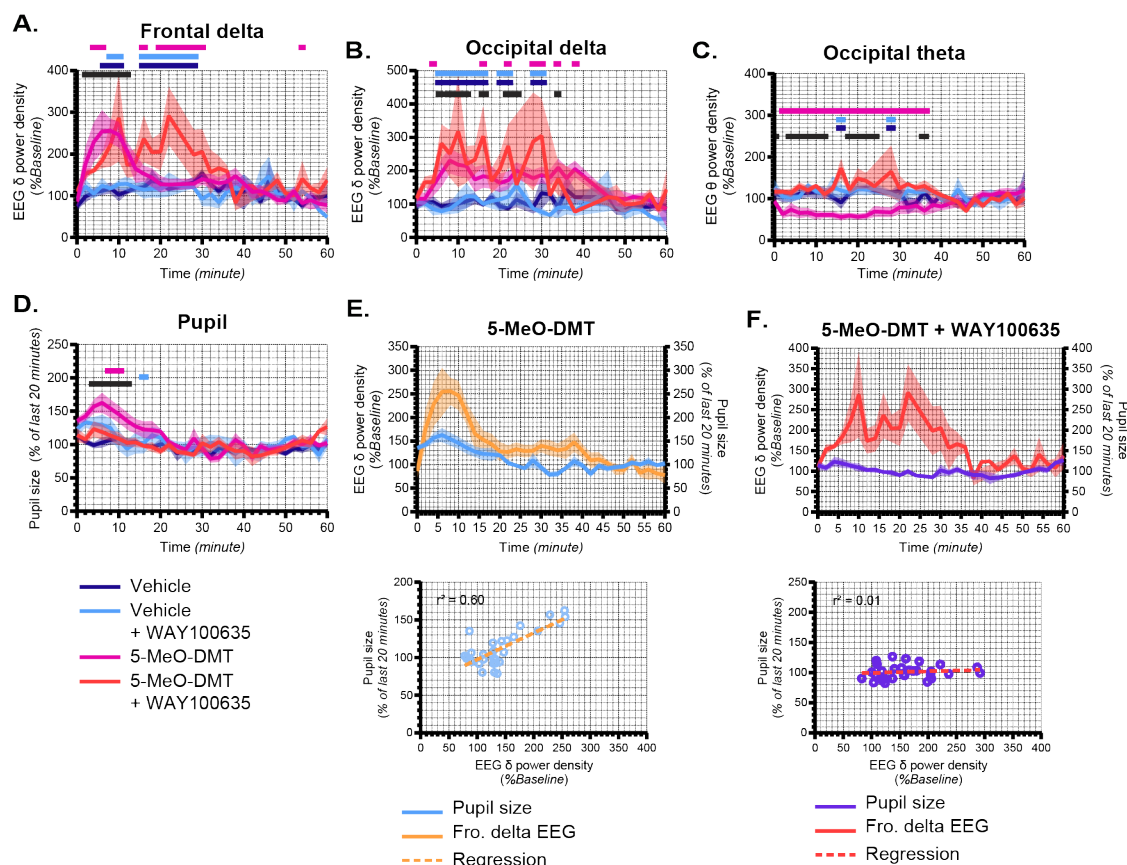
in an increase of SWA compared to vehicle and vehicle+WAY (Fisher's LSD  $p < 0.05$ ), it also resulted in a larger increase of power in slow frequencies than with 5-MeO-DMT alone with two apparent peaks, one at 1.5 Hz and one between 3 Hz and 4.75 Hz (Fisher's LSD  $p < 0.05$ ) (Figures 6.6A).

In the occipital derivation, it was found that vehicle+WAY had largely the same effect as vehicle alone, except for a significant decrease of EEG power between 6.5 – 7 Hz (ME analysis, frequency\*condition:  $F_{234,651} = 13.31$ ,  $p < 0.0001$ , Fisher's LSD  $p < 0.05$ , 5-MeO-DMT against vehicle) (Figure 6.6.B). The 5-MeO-DMT condition significantly increased the levels of delta activity between 0.75 Hz and 4.75 Hz and reduced theta activity between 6.25 Hz and 9 Hz, as reported previously (Fisher's LSD  $p < 0.05$ , 5-MeO-DMT against vehicle) (Figure 6.6.B). As in the frontal derivation, 5-MeO-DMT+WAY increased delta activity compared to vehicle and vehicle+WAY conditions, but also compared to 5-MeO-DMT alone (Fisher's LSD  $p < 0.05$  5-MeO-DMT+WAY against all other conditions) (Figure 6.6.B). In addition,

we found the 5-MeO-DMT+WAY resulted in a higher theta activity compared to 5-MeO-DMT, significantly different from 5-MeO-DMT alone between 6.5 Hz and 7.75 Hz (Fisher's LSD  $p < 0.05$ ), and different from vehicle+WAY only between 7.75 Hz and 8.25 Hz (Fisher's LSD  $p < 0.05$ ). 5-MeO-DMT+WAY theta activity was not completely back to vehicle-levels, but it was different from vehicle alone only between 6.75 Hz and 8.5 Hz (Fisher's LSD  $p < 0.05$ ). These results thus suggest an influence of the 5-HT<sub>1A</sub> receptor in the suppression of theta power by 5-MeO-DMT.

These experiments were also conducted with animals implanted with electrodes in the auditory cortex. In these animals, the vehicle injection coupled with WAY resulted in an increase of intracortical slow activity between 1.5 Hz and 4 Hz (RM-2-way ANOVA frequency\*condition  $F_{234,468} = 5.58$ ,  $p < 0.0001$ , Fisher's LSD  $p < 0.05$  vehicle against vehicle+WAY) (Figure 6.6.C). The injection of 5-MeO-DMT elicited a significant activity increase of power in slow frequencies between 0.75 Hz and 5.5 Hz compared to vehicle alone, as well as a decrease of activity in the theta frequency band between 6.5 Hz and 9.25 Hz (Fisher's LSD  $p < 0.05$  5-MeO-DMT against vehicle) (Figure 6.6.C). The injection of 5-MeO-DMT+WAY also significantly increased activity in the delta frequency bands compared to both vehicle conditions (Fisher's LSD  $p < 0.05$ , 5-MeO-DMT+WAY against vehicle and vehicle+WAY), to a level comparable with the injection of 5-MeO-DMT alone. However, it restored theta activity back to the levels observed after vehicle, rendering it significantly higher than the theta activity in the 5-MeO-DMT alone condition (Fisher's LSD  $p < 0.05$ , 5-MeO-DMT+WAY against 5-MeO-DMT) (Figure 6.6.C). An increase of power in the frequencies  $> 10$  Hz in the 5-MeO-DMT+WAY conditions compared to all other conditions was also noted.

The dynamics of frontal and occipital delta and occipital theta EEG activity were then analysed. As it was shown in Chapter 3 that the dynamics of pupil diameter were correlated to 5-MeO-DMT-induced frontal delta activity pupil diameter were also measured and compared to frontal delta activity. Frontal delta activity remained at the same level in the first 60 minutes of recordings following an injection of vehicle



**Figure 6.7: Time course of EEG powers and pupil dilation in response to a 5-HT<sub>1A</sub> antagonist.** A-D. Time course of the frontal delta (A), occipital delta (B), theta (C) activity, or pupil size (D) during wake episodes after an injection of vehicle, 5-MeO-DNT, vehicle+WAY, or 5-MeO-DMT+WAY. A red, black, pink, or dark or light blue horizontal line denotes a significant difference the two vehicle conditions (red, bottom), 5-MeO-DMT alone condition and the vehicle condition (black), between the 5-MeO-DMT+WAY condition and vehicle condition (dark blue), between the 5-MeO-DMT+WAY condition and vehicle+WAY condition (light blue), and the two 5-MeO-DMT conditions (pink, top) (Fisher's LSD  $p < 0.05$ ). E. Time course of frontal EEG delta activity power and pupil size following an injection of 5-MeO-DMT (top) and correlation of frontal EEG delta activity power and pupil size with regression line (Simple linear regression  $r^2 = 0.23$ ; bottom). F. Time course of frontal EEG delta activity power and pupil size following an injection of 5-MeO-DMT+WAY (top) and correlation of the frontal EEG delta activity power and pupil size with regression line (Simple linear regression  $r^2 = 0.071$ ; bottom). All data are mean + SEM.  $N = 9$  for panels A-C;  $n = 6$  for panels D-F. See Table D.8 & Table D.9 for statistical analyses.

or vehicle+WAY. After the injection of 5-MeO-DMT, an increase of about 250% in the first 10 minutes was noted (ME analysis, time point\*condition:  $F_{84,196} = 2.37$ ,  $p < 0.0001$ ; Fisher's LSD  $p < 0.05$ ) (Figure 6.7.A). The injection of 5-MeO-DMT+WAY also elicited a significant increase of delta power compared to vehicle, and compared to vehicle+WAY at the same time, with a significant difference compared to vehicle from 6 minutes to 10 minutes and vehicle+WAY from 8 minutes to 10 minutes (Fisher's LSD  $p < 0.05$ ), later than 5-MeO-DMT alone (Fisher's LSD  $p < 0.05$ ). However, a second increase occurred later in the 5-MeO-DMT+WAY condition, starting at the 15 minutes mark and culminating with a significant difference of about 300% between 25 minutes and 30 minutes, well higher and later than in all three other conditions (Fisher's LSD  $p < 0.05$ ).

For the occipital delta, the injection of 5-MeO-DMT, with or without WAY, significantly increased delta activity within the first 5 minutes compared to vehicle and remained above 200% until 40 minutes after the injection (ME analysis, time point\*condition:  $F_{78,116} = 2.01$ ,  $p < 0.001$ ; Fisher's LSD  $p < 0.05$ ) (Figure 6.7.B). Only a few random differences were found between 5-MeO-DMT and 5-MeO-DMT+WAY.

For the occipital theta both vehicle conditions remained equal (ME analysis, time point\*condition  $F_{87,143} = 2.92$ ,  $p < 0.0001$ ; Fisher's LSD  $p > 0.05$  between both vehicle conditions) (Figure 6.7.C). 5-MeO-DMT alone reduced theta activity to 70% of baseline, with significant differences from vehicle occurring between 2 – 25 minutes and at around 35 minutes post-injection (Fisher's LSD  $p < 0.05$ ) (Figure 6.7.C). It was found that 5-MeO-DMT+WAY restored theta activity to vehicle levels, with theta activity being significantly higher than in the 5-MeO-DMT alone conditions (Fisher's LSD  $p > 0.05$ ) (Figure 6.7.C).

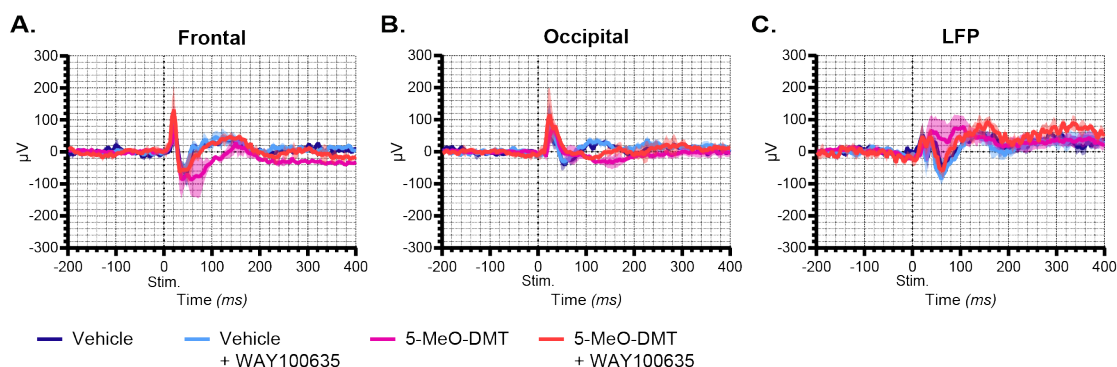
Regarding pupil diameter, it was found that neither vehicle nor vehicle+WAY had any effect, and that the pupil diameter remained stable throughout the recordings (ME analysis, time point\*condition  $F_{120,401} = 1.64$ ,  $p < 0.001$ ; Fisher's LSD  $p > 0.05$ ) (Figure 6.7.D). The injection of 5-MeO-DMT increased pupil diameter, nearing 170% dilation within 5 minutes, and it remained significantly different from

vehicle from 4 minutes to 12 minutes before returning to vehicle-like levels of dilation (Fisher's LSD  $p < 0.05$ ) (Figure 6.7.D). 5-MeO-DMT coupled with WAY, however, did not follow such pattern and pupil diameter remained comparable to vehicle throughout the recording session (Fisher's LSD  $p > 0.05$ ), so much so that it was significantly less dilated than in the 5-MeO-DMT alone condition from 8 minutes to 10 minutes (Fisher's LSD  $p < 0.05$ ) (Figure 6.7.C). These results suggest that the increase of pupil diameter induced by 5-MeO-DMT is mediated by 5-HT<sub>1A</sub> receptors.

Chapter 4 showed that the mydriasis induced by 5-MeO-DMT was correlated with frontal EEG delta power, and this was still the case in this set of experiments (Pearson's simple linear regression  $r^2 = 0.60$ ,  $p < 0.05$ ) (Figure 6.7.D). However, in the 5-MeO-DMT+WAY condition, pupil diameter and frontal delta activity had different dynamics. Delta dynamics were slower and more intense, as described previously, while pupil diameter remained relatively stable throughout, leading to a lack of correlation (Pearson's simple linear regression  $r^2 = 0.01$ ,  $p = 0.54$ ). As the time course of delta activity changes with WAY, it is possible to infer that some of the processes underlying the regulation but not the generation of slow waves by 5-MeO-DMT are mediated by 5-HT<sub>1A</sub> receptors, leading to a correlation with pupil diameter.

### 6.3.4 5-HT<sub>1A</sub> antagonist affects auditory ERPs

To investigate the role of 5-HT<sub>1A</sub> receptors in the alteration of auditory-induced ERPs by 5-MeO-DMT, the auditory stimulation protocol was conducted in a group of animals who also received the WAY-100635 antagonist with 5-MeO-DMT or vehicle. As in Chapter 5, a RM-2-way ANOVA with Greenhouse-Geisser's correction did not reveal any significant difference between conditions or time, nor any interaction in the EEG frontal derivation (RM-2-way ANOVA time point\*condition:  $F_{459,459} = 1.81$ ,  $p = 0.41$  (GG)) or in the occipital derivation (RM-2-way ANOVA time point\*condition:  $F_{459,459} = 2.20$ ,  $p > 0.38$  (GG)), as well as in the LFP signals



**Figure 6.8: ERPs in response to 5-MeO-DMT injection and the presence of a 5-HT<sub>1A</sub> receptor antagonist.** A-B. ERP in the frontal (A) and occipital (B) EEG derivations during artefact-free episodes of waking following an injection of vehicle, 5-MeO-DMT, vehicle+WAY or 5-MeO-DMT+WAY. C. ERP in the LFPs during artefact-free episodes of waking following an injection of vehicle, 5-MeO-DMT, vehicle+WAY or 5-MeO-DMT+WAY. N = 3 for all panels. See Table D.10 for statistical analyses.

(RM-2-way ANOVA time point\*condition  $F_{459,918} = 1.63$ ,  $p = 0.31$ ) (Figure 6.8).

However, visual inspection of the signal showed that, in the frontal derivation, the injection of 5-MeO-DMT+WAY resulted in ERPs similar to an injection of vehicle. The injection of 5-MeO-DMT alone tends to introduce a wider N40 and a slight delay in the expression of P120. Administration of WAY however produced an ERP with a N40 and P120 components visibly occurring at the same time and amplitude as the one following an injection of vehicle (Figure 6.8.A). This was not the case in the occipital derivation, where 5-MeO and 5-MeO-DMT+WAY induced a similar response, with a negative wave occurring instead of P120 and a total absence of N40, both normally observed in the vehicle and vehicle+WAY condition (Figure 6.8.B). However, as no significant difference was found, interpretation must be made with caution.

ERPs occurring after an injection of vehicle showed a biphasic early positive response in intracortical recordings, followed by a waning trough-and-wave pattern suggesting an N40 and P120 components (Figure 6.8.C). 5-MeO-DMT injection, however, showed only a large positive wave from 20 – 200 ms post-stimulus, with no sign of an N40 or P120 component, as presented in Chapter 5. In the 5-MeO-DMT+WAY condition, the overall shape of the ERP was similar to the vehicle



condition (Figure 6.8.C). This can lead to the idea that the 5-HT<sub>1A</sub> receptor might be implicated in the signal modulation properties of 5-MeO-DMT at least in frontal cortical areas and the parietal cortex. However, as above, as no significant difference was found, interpretation must be made with caution.

Isolation of the individual components as conducted in Chapter 5 was conducted and is presented in Appendix D, section D.2.

Taken together, these results point towards different effects of WAY on EEG ERP components. While WAY might seem to counteract some alteration from 5-MeO-DMT, it also had some significant effect when coupled with vehicle.

## 6.4 Discussion

### 6.4.1 5-MeO-DMT-slow waves and sleep homeostasis

The injection of 5-MeO-DMT following sleep deprivation showed that 5-MeO-DMT increased slow wave activity during the initial period of wakefulness following the injection. Sleep deprivation was successful at increasing SWA during the subsequent episode of SWS in vehicle animals. However, it failed to also further increase the amplitude of slow waves induced by the injection of 5-MeO-DMT during the initial wakefulness, with a potential decrease of the lowest frequencies of the delta range in the occipital derivation. These results suggest that sleep pressure has little to no effect on the intensity of the 5-MeO-DMT-generated slow waves. This further supports the notion that slow waves can be of different origin and may have somewhat different mechanisms depending on state, but also raises the possibility that the bistable neuronal state, while normally impacted by sleep regulatory processes, can also occur independently. However, the possibility remains that there exists a set maximum level of slow wave activity during wake which was already reached after the administration of 5-MeO-DMT, thus further increase of slow wave activity by sleep deprivation was prevented by a ceiling effect.

Regarding the dynamics of frontal and occipital delta EEG power, the sleep deprivation experiment did reproduce previous results for delta activity in the frontal

and occipital derivations visually but not statistically. Indeed, in both derivations, a visual increase of delta power was noted immediately following an injection of 5-MeO-DMT regardless of sleep deprivation. It is possible that circadian processes might impact the generation of slow waves by 5-MeO-DMT. The injections were made at ZT4 and not ZT0. Knowing that the behavioural effects of 5-MeO-DMT and sleep processes are both influenced by the circadian rhythm (Borbély, 1982; Moser & Redfern, 1985), therefore is plausible that slow waves generated by 5-MeO-DMT are influenced as well by circadian processes, with injections closer to ZT0 leading to higher slow wave activity than ZT4 injections. However, as the rate of HTR is stable over the dark period but follows an inverted U shape during the light period (Moser & Redfern, 1985), it might have been expected to see an increase in 5-MeO-DMT generated slow waves and not a hypothetical decrease. This lack of increase however result from a ceiling effect described earlier preventing 5-MeO-DMT-slow waves to reach a higher activity. Moreover, sleep deprivation is known to induce slow wave during wake episodes Vyazovskiy et al. (2011a, 2011b). Therefore, vehicle delta activity might have been slightly elevated, preventing the detection of a significant difference between vehicle and 5-MeO-DMT conditions.

Another argument to tentatively explain this lack of difference between the drug and vehicle conditions would be a change of set and setting, a concept used to describe the context-dependant effect of psychedelics (Carhart-Harris et al., 2018a; Pollan, 2019). During sleep deprivation, the nests were removed from the cages before being re-introduced in the environment at the end of sleep deprivation, upon the injection of 5-MeO-DMT. It is highly common for mice to immediately proceed to repair their nest. This strong change of context might lead to the downsizing of psychedelic effect as hypothesised in Chapter 3 subsection 3.4.2.

The overall dynamics of theta activity were not altered, however, and remained lower than vehicle in both 5-MeO-DMT conditions, suggesting that the effects of 5-MeO-DMT on the theta rhythm were unchanged by sleep deprivation and the change of injection time, or context.

Further analysis focussed on sleep shows that sleep deprivation coupled with 5-MeO-DMT successfully increased slow wave activity to a higher level than 5-MeO-DMT alone, which remained at the same rate as vehicle during SWS. However, during SWS 5-MeO-DMT alone induced a reduction of frontal EEG activity from 7 Hz onwards compared to vehicle alone, a phenomenon not observed in the animals subjected to sleep deprivation. In the occipital derivation, a slight decrease in EEG power was observed in the 5-MeO-DMT condition for frequencies around the theta band, suggesting that the animals entered sleep while the suppression of the theta rhythm was still occurring.

As this was also not observed in the animals of previous chapters, this could be the result of the decreased latency to enter SWS. Indeed, while 5-MeO-DMT alone successfully delayed the occurrence of SWS and PS, the increased sleep pressure allowed an earlier SWS onset when sleep deprivation was coupled with 5-MeO-DMT. If we consider the balance between state and that the effects of psychedelics are mostly observable during wake state, the increased sleep pressure might have exerted a greater pull towards the occurrence of SWS, as suggested by the homeostatic regulation of sleep (Borbély, 1982), which in turns might have cut short the effects of 5-MeO-DMT.

It has been shown that limiting the biosynthesis of 5-HT can lead to insomnia without alteration of the markers of sleep pressure (Sallanon et al., 1983; Tobler & Borbély, 1982). The results presented here on sleep architecture support that notion and will be discussed in 6.4.3 in relation to the WAY experiments. It has been reported, however, that the delay in PS reported as a key effect of psychedelic administration on sleep (Barbanoj et al., 2008; Dudysová et al., 2020; Thomas et al., 2022) is not altered by sleep deprivation, if counted from SWS onset. A leading idea is that this suppression of PS is due to an activation of 5-HT receptors, which might mimic a 5-HT-rich environment detrimental to the occurrence of PS. However, as all injections were made at the same time, the occurrence of consolidated PS episode 20 minutes earlier in the case of sleep deprivation, but at the same time after SWS onset suggests otherwise.

Indeed, observation of PS homeostasis shows that the inter-PS interval can be determined by the duration of the previous PS episode, with SWS being considered an inter-PS phase (Vivaldi et al., 1994). In the case of a 5-MeO-DMT injection, the duration between SWS onset and PS onset was similar with or without sleep deprivation, and still significantly longer than after vehicle administration. If this suppression of PS was purely pharmacological, we would have expected PS onset to occur at the same time from the injection, and not at the same time from sleep onset. The results presented here support the notion that the altered state induced by psychedelics, despite the occurrence of slow waves and suppression of theta, might cover some function of PS, which is in line with the notion that both PS and psychedelics share some involvement in the processing of emotional memory (Griffiths et al., 2006; Luppi et al., 2022; Osmond, 1957; Vollenweider & Preller, 2020).

#### 6.4.2 5-MeO-DMT effects might not be inducible locally

The aim of the intracortical injection of 5-MeO-DMT was to investigate whether the local diffusion of 5-MeO-DMT was sufficient to generate bi-stability in the local network. The results presented in this chapter however suggest otherwise. The only notable difference was an increased theta rhythm in the occipital EEG in the 5-MeO-DMT+sleep deprivation, which could simply be explained by the nature of sleep deprivation. In the LFP, while the theta activity was increased, it was also the case for a wide range of other frequencies. As increased theta activity has been associated with exploratory behaviour (O’Keefe & Recce, 1993; Zhan, 2015), and because the electrodes were located in the somatosensory cortex, it is possible that this reflects the increased neuronal activity during tactile exploration similar to the explorations the sleep-deprivation protocol relies on (Fortier-Poisson & Smith, 2016).

The lack of slow waves, however, seems to suggest that the effects of 5-MeO-DMT on the network are not of local origin. Examples of local generation of slow waves and local sleep-like signals in the cortex during episodes of wake do exist

(Vyazovskiy et al., 2009a, 2011a, 2011b), and can also occur following injuries in humans (Sarasso et al., 2020). The results presented here suggest that the effects of 5-MeO-DMT on cortical activity requires a systemic injection or, a minima, may require an intraventricular injection and therefore have a different generative mechanism than local slow waves occurring during wake, thus supporting the idea that 5-MeO-DMT injections induce a brain-wide state rather than alterations of local networks.

Together with the notion that unaltered 5-HT neurotransmission is necessary for sleep onset (M. Jouvet, 1999; Tobler & Borbély, 1982), the generation of slow waves, as observed after the injection of 5-MeO-DMT, might rely on the occurrence of a given state of the network. Indeed, for a neuron to exhibit bistability, it may be required that its surrounding network is also in such a state. This might be the case with local slow wave generation, or with cortical networks surrounding a lesion site disconnected from a wider network, thus allowing the local network to enter a slow wave promoting state (Krone, 2023). However, because the whole network is still highly connected after the intracortical injections, the neurones might not be able to enter a bi-stable state locally. Further experiments with high density recordings might reveal changes in the activity patterns of individual neurons.

This is, however, the first time that such an intracortical infusion experiment has been done with this substance and in mice. The concentrations have been calculated based on the literature in rat and based on DOI (Willins & Meltzer, 1997), by comparing systemic injections of DOI and 5-MeO-DMT (Halberstadt et al., 2012; Mazzola-Pomietto et al., 1995; Riga et al., 2014). It is entirely plausible that the calculated concentration was not high enough, and a higher dose of 5-MeO-DMT might induce local-slow waves and locally suppress theta activity.

The injections were performed in the somatosensory cortex, for which no measurement has been done before. The reason for this change in cortical area follows the observations in Chapter 4 that mice heavily rely on whisker movement

to perform exploratory behaviour, suggesting that mice might rely more extensively on their somatosensory cortex rather than their visual cortical area to explore their environment. Results from Chapters 3 and 4 from the parietal cortex seem to suggest that the slow waves in the LFP are occurring at a global scale, and there was no reason to believe that the somatosensory cortex was immune to the effects of 5-MeO-DMT. We did not, however, have the evidence that 5-MeO-DMT induces slow wave activity in the somatosensory cortex prior to the experiment presented in this chapter, and it is plausible -albeit quite improbable due to the distribution of the 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors which are thought to be responsible of such effects (Nichols & Nichols, 2008)- that the lack of response to intracortical injection is due to the somatosensory cortex itself rather than a network effect or a below-threshold drug concentration. Further dose-dependent experiments are required before expressing stronger conclusions.

### 6.4.3 5-HT<sub>1A</sub> and the effects of 5-MeO-DMT

The effects of *classical* psychedelics are known to be mediated by the 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors (Nichols et al., 2022; O'Donnell et al., 2023). Therefore, these are the perfect starting point for a pharmacological analysis of the effects reported in the previous chapters. The 5-HT<sub>1A</sub> receptor was selected because of the higher affinity of 5-MeO-DMT for it (Halberstadt et al., 2012). WAY is a 5-HT<sub>1A</sub> receptor antagonist shown to actively suppress 5-HT<sub>1A</sub>-associated activity and increase the firing rate of DRN neurons (Forster et al., 1995), with a high solubility in saline allowing a vehicle solution similar to the one used in previous experiments. Importantly, WAY has shown some affinity as an agonist for the dopaminergic D4 receptor, suggesting that some effects reported in this chapter might be due to an activation of the D4 receptors (Chemel et al., 2006).

In this chapter, the injection of vehicle alone, or jointly with WAY, did not elicit any visible changes in terms of behaviour or cortical activity. The only reported change was in the amplitude of the P20 component, which was elevated when WAY

was administered. While this could be linked to an activation of the D4 receptors, previous studies on humans failed to report any effect of dopaminergic activation on auditory ERPs (O'Neill et al., 2006).

The first observation made with the injection of WAY, coupled with 5-MeO-DMT, was behavioural. Indeed, despite not being formally quantified, all animals injected with 5-MeO-DMT+WAY exhibited an exacerbated behavioural response compared to 5-MeO-DMT alone, more akin to what has been described with LSD or psilocin (Thomas et al., 2022). Indeed, immediately after the injection of 5-MeO-DMT+WAY, the animals showed less locomotor behaviour and spent most of their time in the nest. Not many exploratory attempts were made and HTR seemed increased and exaggerated. Such observations have already been reported in previous experiments with DOI, a psychedelic and potent 5-HT<sub>2A</sub> receptor agonist (Willins & Meltzer, 1997). Indeed, in their experiment, the authors injected WAY along with DOI and reported an increase in HTR, already established as a key behavioural marker of 5-HT<sub>2A</sub> activation. They suggested that the 5-HT<sub>1A</sub> autoreceptor, thanks to its hyperpolarisation properties, might be able to inhibit the 5-HT<sub>2A</sub> excitatory responses, hence why a 5-HT<sub>1A</sub> inhibitor might release that inhibition and enhance the behavioural response to 5-HT<sub>2A</sub> activation.

The injection of WAY coupled with 5-MeO-DMT enhanced slow wave activity during the first 30 minutes following the injection in both derivations. Delta dynamics over this period in the frontal derivation showed a different response, with an initial slow increase reaching levels similar to the 5-MeO-DMT response but continuing over 25 minutes towards a larger increase. When considering pupil diameter, the addition of WAY prevented the increase in pupil size observed with 5-MeO-DMT injections alone. This experiment reproduced previous results and found a correlation between pupil diameter and delta activity in the case of 5-MeO-DMT injections alone.

However, when coupled with WAY, the observed slow wave dynamics did not occur jointly with pupil diameter increase. While these results show that slow wave

generation following 5-MeO-DMT administration is not mediated by 5-HT<sub>1A</sub>, it is possible that the mechanism underlying the fast decrease in frontal delta activity also observed with pupil diameter might rely on 5-HT<sub>1A</sub> activation. However, the larger increase in delta activity observed with 5-MeO-DMT+WAY suggests that the existence of other mechanisms capable of generating slow waves and not relying on pathways leading to increased pupil diameter. However, as D4 receptors are also activated by WAY (Chemel et al., 2006), and because they have been shown to be involved in light adaptation and arousal mechanisms in rodents (Flood & Eggers, 2021; Nakazawa et al., 2015), we cannot rule out 5-HT independent mechanisms at play.

Regarding theta activity in the occipital EEG and LFP derivations, the addition of WAY to 5-MeO-DMT administration, prevented the theta suppression occurring with 5-MeO-DMT injections. This suppression is therefore likely mediated by the 5-HT<sub>1A</sub> receptor. These receptors are known to induce a hyperpolarisation of 5-HT containing neurons of the DRN, but their localisation in the hippocampus suggests potential similar effect on hippocampal neurons (Pazos et al., 1985; Sharp et al., 2007). The theta rhythm is partly mediated by the medial septal nucleus of the hippocampal formation (Boyce et al., 2016). It is therefore possible that an activation of the 5-HT<sub>1A</sub> receptor by 5-MeO-DMT further disrupts neuronal activity in this hippocampus and prevents the physiological generation of the theta rhythm.

On auditory evoked responses, WAY might have restored the latency and aspects of the continuous ERPs, with a vehicle-like P20, N40 and P120, in the frontal EEG derivation and LFP. It altered the amplitude and latency of some specific components but not all. The main effect consisted of the P120 being restored to vehicle levels in the EEG frontal derivation, and restoration of a positive P120 in the EEG occipital derivation. Taken together, these results suggest that alteration of ERPs by 5-MeO-DMT are partially mediated by the 5-HT<sub>1A</sub> receptor. These alterations concern P120 which has been linked to cortical control of the ERPs in



relation to sustained-attention and novelty (Muller-Gass & Campbell, 2019). WAY, however, did not reduce the amplitude of the P20 and N40 towards vehicle-like levels. Amplitudes of P20 and N40 have been suggested to be altered by 5-HT<sub>2A</sub> (Maxwell et al., 2004). The results presented here also go in that sense. It is plausible that WAY successfully prevented most of 5-MeO-DMT to bind to 5-HT<sub>1A</sub> receptor, which then activated more 5-HT<sub>2A</sub> receptors.

The inhibition of the 5-HT<sub>2A</sub> neurotransmission by 5-HT<sub>1A</sub> argued by Willins and Meltzer (1997) might also help explain these observations. Indeed, as WAY with vehicle enhanced P20, a further elevation of P20 amplitude observed in the 5-MeO-DMT+WAY condition might result from an over-activation of the 5-HT<sub>2A</sub> receptors. However, this does not explain the changes in N40 amplitude, other than they do not rely on 5-HT<sub>1A</sub> neurotransmission despite being altered by 5-MeO-DMT. These alterations might result from other downstream effects.

The limitations of the analysis of specific ERP elements like the one conducted here are the same as the ones presented in Chapter 5. Indeed, while the nature of the fitted curved provided a better detection of the ERP elements, it might have underestimated changes in the amplitude. Therefore, analysis of the shape of the ERPs might provide a better understanding on the pharmacology underlying the potential alteration of ERPs by 5-MeO-DMT. If that is the case, our result suggest that 5-HT<sub>1A</sub> receptor might be at least partially responsible for a disruption of ERPs caused by 5-MeO-DMT. However, stronger analyses are required.

#### 6.4.4 Conclusion

This chapter investigated potential regulatory mechanisms underlying the changes in behaviour and brain activity observed after an injection of 5-MeO-DMT. Three different approaches were used, one relying on sleep deprivation, one on intracortical infusions, and one on the injection of a 5-HT<sub>1A</sub> antagonist.

The sleep deprivation protocol suggested that the slow wave activity measured after an injection of 5-MeO-DMT is unlikely to be relying on sleep mechanisms,

as changes in sleep/wake history did not alter their amplitude despite successfully increasing the amplitude of slow waves during sleep after sleep deprivation.

The results from intracortical injections of 5-MeO-DMT suggest that the alterations measured in this chapter and previous chapters are more likely to be a global phenomenon rather than a local alteration of the network, but further studies with elevated concentrations of 5-MeO-DMT are required.

Finally, the injection of a 5-HT<sub>1A</sub> antagonist seems to have enhanced some behavioural responses commonly associated with the activation of 5-HT<sub>2A</sub> receptors and other psychedelics like LSD or psilocybin, further supporting the notion that an inhibitory interaction between 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor exists. Furthermore, the results presented here also show that the generation of slow wave following an injection of 5-MeO-DMT is not relying on 5-HT<sub>1A</sub> activation. However, while the effects imbued to psychedelics in humans and rodents might be mediated by the 5-HT<sub>2A</sub> receptor, the activation of 5-HT<sub>1A</sub> receptor is likely to play an important modulatory role as they are involved in the suppression of the theta rhythm observed after administration of 5-MeO-DMT.

# 7

## General discussion

### Contents

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|                                                                                                   |            |
|---------------------------------------------------------------------------------------------------|------------|
| <b>7.1 Summary . . . . .</b>                                                                      | <b>194</b> |
| <b>7.2 A proposed circuitry of 5-MeO-DMT activation. . . .</b>                                    | <b>197</b> |
| 7.2.1 The activation of 5-HT <sub>1A</sub> modulates cortical activity . .                        | 197        |
| 7.2.2 The interaction between 5-HT <sub>1A</sub> and 5-HT <sub>2A</sub> . . . . .                 | 200        |
| <b>7.3 Exploring the delay of paradoxical sleep . . . . .</b>                                     | <b>202</b> |
| <b>7.4 Slow waves as a mechanism supporting sleep and<br/>    therapeutic processes . . . . .</b> | <b>205</b> |
| 7.4.1 Slow wave activity and the recovery processes of sleep .                                    | 205        |
| 7.4.2 The therapeutic properties of slow waves . . . . .                                          | 207        |
| <b>7.5 A brain-wide state for psychedelic therapeutic action .</b>                                | <b>209</b> |
| <b>7.6 Conclusion . . . . .</b>                                                                   | <b>211</b> |

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Psychedelic drugs such as LSD, psilocybin and 5-MeO-DMT are known to induce an altered state of consciousness in humans, and recent studies demonstrated that they also have therapeutic properties against major mental illnesses such as anxiety and treatment-resistant depression (Rucker et al., 2018). Furthermore, it has been shown that the effect of these drugs was context-dependent in humans, with the content of the experience being linked to positive outcomes on mental health (Carhart-Harris et al., 2018a, 2018b). The underlying mechanisms are thought to be mediated by 5-HT<sub>2A</sub> activation, as these receptors are known to induce the psychedelic experience in humans and the equivalent head twitch response in

mice (González-Maeso et al., 2007; Vollenweider et al., 1998). Importantly, sleep disruption is a common symptom of those illnesses. However, surprisingly, the study of psychedelic's effects on sleep and wake state has received very little attention. The main aim of this work was to address this omission.

Psychedelic drugs are known to alter serotonergic neurotransmission, which is strongly involved in the regulation of wake and sleep states. For example, pharmacological alteration of the serotonergic system with antidepressants like selective-serotonin reuptake inhibitors (SSRIs) or monoamine oxidase inhibitors (MAOIs) has been shown to actively suppress the occurrence of paradoxical sleep without clear rebound (Wichniak et al., 2017). We therefore argue for the possibility that the therapeutic properties of psychedelic result from an altered state of vigilance.

The main aim of this project was to investigate the potential changes induced by the short-lasting psychedelic 5-MeO-DMT on brain activity and behaviour across sleep and wake states in laboratory mice. Such changes were expected to provide novel insights into the potential therapeutic properties of psychedelics.

## 7.1 Summary

The first Results chapter of this thesis presented the effects of a systemic injection of 5-MeO-DMT on cortical activity and behaviour in mice. It was observed that 5-MeO-DMT induced head-twitch response (HTR), and decreased food-seeking behaviour, but did not markedly affect the animal's motor control or its ability to explore its environment. The brain activity during wakefulness was, however, markedly affected, with cortical activity showing an activity pattern closer to sleep in appearance, with a global increase in slow wave activity and a suppression of the theta rhythm in occipital areas. The increase of frontal and occipital delta activity followed the same dynamic as the suppression of the occipital theta activity. Intracortical recordings also revealed that the slow waves also occurred in LFP recordings and were accompanied by neuronal off periods, as in NREM sleep. In terms of the

sleep/wake cycle, the injections of 5-MeO-DMT delayed sleep onset and induced a further delay in PS latency but did not alter cortical activity during these states.

Then, the next chapter focussed on measurements of attention and arousal. The aim of this work was to explore further the qualitative characteristics of the hybrid vigilance state induced by psychedelics. The behavioural task we have chosen appeared to be not ideal for our purposes and was not pursued further. Instead, we invested time in the development of a novel device to measure pupil dilation and eye movement in freely behaving rodents. This is typically done in head-fixed animals which arguably can affect behaviour and arousal, and therefore we thought it is essential for such a paradigm to be performed in unrestrained rodents. This experiment revealed that 5-MeO-DMT injections were followed by a dramatic increase in pupil size, suggesting elevated arousal levels in the animals. Jointly with EEG recordings, this chapter also suggested that this increase in pupil size was paradoxically correlated with the increased frontal delta activity and theta suppression, which are typically considered as features of decreased cortical arousal. This chapter also showed that frontal delta dynamics were somewhat different from occipital delta and theta dynamics, which were longer lasting. Concerning pupil dilation changes, they were shorter lasting and more closely correlated with frontal delta activity.

The next series of experiments aimed to investigate state-dependent and 5-MeO-DMT-dependent alterations of evoked response potentials (ERPs) to auditory stimulation. It was found that the overall shape of ERPs was conserved across vigilance states, but the ERPs showed greater and more elongated responses during sleep. The injection of 5-MeO-DMT somewhat altered later ERP components, such as the N40 and P120 components, in both EEG and intracortical LFP recordings. This chapter also provided further confirmation of a different cortical dynamics between frontal and occipital cortical areas. We interpret as the former being more context dependent, due to a shortening of the frontal effects only observed when strong changes in the environment are presented such as auditory stimulation or the wearing of an oculometre.

The final chapter aimed to investigate potential mechanisms responsible for, or capable of modulating, the reported effects of 5-MeO-DMT. It was first shown that sleep deprivation did not further increase the 5-MeO-DMT-induced slow wave activity. However, it lowered the latency to sleep onset and PS onset by the same amount, suggesting that 5-MeO-DMT affects PS homeostatic regulation rather than merely suppressing it. It was also shown that an intracortical application of 5-MeO-DMT was not sufficient to elicit any previously reported changes, suggesting that brain-wide injections of 5-MeO-DMT are needed, and supporting the notion that the effects on local field potentials and multi-unit activity reported previously likely result from brain-wide dynamics. Finally, the use of a 5-HT<sub>1A</sub> receptor antagonist WAY-100635 (WAY) provided important evidence that both the suppression of the theta rhythm and increase of pupil diameter induced after an injection of 5-MeO-DMT are mediated by an activation of 5-HT<sub>1A</sub> receptors, as well as changes in intracortical and frontal ERPs. Furthermore, the inhibition of 5-HT<sub>1A</sub> receptors failed to restore occipital ERPs, and a large increase in frontal and occipital delta was still observed. However, the addition of WAY did affect frontal delta-activity dynamics, manifested in an increase, consistent with the one observed in the occipital derivation, suggesting that the suppression of the frontal delta-activity back to vehicle-like levels reported in previous chapters might be mediated through 5-HT<sub>1A</sub> receptors.

Overall, this thesis showed that 5-MeO-DMT administration acutely alters behaviour, brain activity and sleep architecture by producing an altered wake state with clear markers of wakefulness, such as motor activity, exploration, and elevated arousal accompanied with, paradoxically, brain markers of sleep and decreased cortical arousal. While it is unclear whether this altered state corresponds to the psychedelic state in humans, the presence of large, global slow waves could potentially confer it with some of the restorative properties of sleep. At the same time, its suppression of paradoxical sleep suggests that this state might express at least some of the hypothesised function of paradoxical sleep and therefore could be

implicated in emotional regulation processes and mental health.

## **7.2 A proposed circuitry of 5-MeO-DMT activation.**

### **7.2.1 The activation of 5-HT<sub>1A</sub> modulates cortical activity**

With the previous results chapters, it is possible to establish a rudimentary description which tentatively explains the effects of 5-MeO-DMT on brain activity and behaviours (Figure 7.1). Importantly, all experiments with systemic injections of 5-MeO-DMT showed that a single injection acutely increases frontal and occipital EEG delta activity and pupil diameter. We showed that the increase in pupil diameter was correlated with the frontal delta activity but not the dynamics in the occipital derivation, which outlasted the effects on the pupil. The injections of 5-MeO-DMT also strongly suppressed theta activity in the occipital derivation. The injection of a 5-HT<sub>1A</sub> antagonist prior the injection of 5-MeO-DMT prevented the suppression of the theta activity and the increase of pupil diameter. Thus, we conclude that the theta suppression and pupil mydriasis observed following 5-MeO-DMT injections are due to 5-HT<sub>1A</sub> activation.

It was also observed that the frontal delta activity had a different dynamic across different experiments, with an increase lasting longer when the animals were left undisturbed. This increase had a similar time-course in the occipital derivation. However, contextual changes, i.e. when the animals were wearing the oculometre or when they were subjected to auditory stimulation, led to more transient frontal delta-activity increase, unlike in the occipital derivation which showed delta and theta activity changes that were not affected by the context.

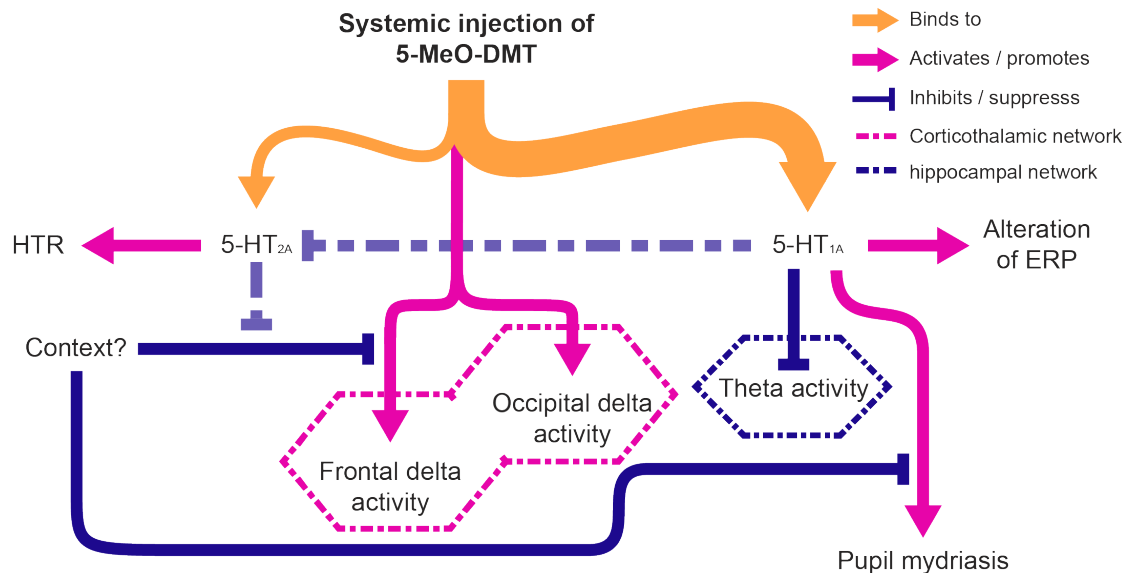
We hypothesise that changes in the environment are responsible for the lack of sustained increase in frontal slow wave activity in the latter experiments. The observation of slow wave activity during wakefulness in rodents is mostly confined to the concept of “local sleep” as illustrated by intracortical recordings of rats showing the spontaneous occurrence of slow waves during a prolonged state of wake

(Vyazovskiy et al., 2011b). In humans, the observation of global EEG slow wave activity in an altered state has been reported before in sleep-walking patients or around lesion sites (Idir et al., 2022; Sarasso et al., 2020), but very little is known about the modulation of slow waves during the wake state. Therefore, further work is required to distinguish both intrinsic, pharmacological and neurophysiological, and extrinsic, contextual and environmental, mechanisms underlying the dynamics of slow-wave activity, and our data provide new important insights.

In Chapter 1, it was argued that cortical slow waves could originate from layer V pyramidal cells but that cortico-thalamic networks were responsible for their modulation and maintenance (David et al., 2013; Krone et al., 2021; Sanchez-Vives & McCormick, 2000). A selective excitation of thalamocortical neurons in rats has been shown to entrain EEG slow waves (David et al., 2013). The thalamus is considered an essential actor in sensorial input processing as a relay from primary sensory inputs to the cortex (McCormick & Bal, 1994; Purves, 2012). Thalamic neurons are also hyperpolarised during sleep, resulting in a “thalamic gating” thought to be important in maintaining sleep stability and slow wave integrity (McCormick & Bal, 1994; Steriade et al., 1993a).

Moving from the thalamus, Chapter 5 reports studies suggesting that 5-HT<sub>2A</sub> receptors may be responsible for the modulation of the cortical gating of sensory inputs. Specifically, the administration of 5-HT<sub>2A</sub> agonist olanzapine was shown to increase late-ERP components which can be interpreted as an inhibition of cortical gating processes (Maxwell et al., 2004). We can speculate however that the mechanism underlying the faster return to vehicle-levels of delta activity observed in the frontal derivation following the initial 5-MeO-DMT-induced increase results directly from the thalamocortical or cortico-cortical signal-modulation circuitry akin to cortical gating-processes.





**Figure 7.1: A summary of the effects of 5-MeO-DMT on brain activity mediated by 5-HT neurotransmission.** Chapter 3 showed that a systemic injection of 5-MeO-DMT increased the head twitch response (HTR), increased frontal and occipital slow wave activity and suppressed occipital theta activity. Chapter 4 showed that the same injection induced pupil mydriasis with the same dynamics as frontal delta activity. It also showed that the increase of frontal delta activity had a different dynamics than the occipital delta and the occipital theta. Chapter 5 suggested that a systemic injection of 5-MeO-DMT altered evoked response potentials (ERPs) to auditory stimulation. It also showed a faster decay of frontal delta increase, similar to the one observed in Chapter 4 suggesting an influence of context on frontal delta activity, and therefore on pupil mydriasis. Chapter 6 showed that the 5-MeO-DMT-mediated increase in frontal and occipital delta are unlikely to be mediated by sleep processes, and that only a systemic injection of 5-MeO-DMT induced change in local and global activity patterns. It also showed that the 5-HT<sub>1A</sub> receptor mediated the suppression of the occipital theta activity, pupil mydriasis and the alteration of auditory ERPs. It suggested also that 5-HT<sub>1A</sub>, by inhibiting 5-HT<sub>2A</sub> might modulate the context sensitivity of frontal delta increase. In dash lines are represented putative brain regions. These are speculations and further experiments are required to determine the source of the observed changes in brain activity patterns.

### 7.2.2 The interaction between 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub>

5-MeO-DMT is a non-selective 5-HT receptor agonist and has highest affinity for the 5-HT<sub>1A</sub> receptor, 300-fold higher than its affinity for the 5-HT<sub>2A</sub> receptor (Halberstadt et al., 2012). After a systemic injection, 5-MeO-DMT binds to 5-HT<sub>1A</sub> receptors, but also to 5-HT<sub>2A</sub> receptors as shown by the occurrence of head twitch responses (HTR), a 5-HT<sub>2A</sub>-mediated behaviour regarded as an expected response to psychedelic intoxication in rodents (González-Maeso et al., 2007). However, in the presence of WAY, we observed an increase in HTR, a lower mobility of the animal and full-body tremors. As discussed in Chapter 6, this increase of HTR following the injection of WAY has already been reported after intracortical infusion of DOI in rats (Willins & Meltzer, 1997). Injections of increasing concentrations of WAY in rats were sufficient to elicit HTR, but not if it was preceded with the injection of the 5-HT<sub>2A</sub> selective antagonist SR 46349B (Darmani, 1998). These results led to the assumptions of a reciprocal interaction between 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors. A study conducted on knock-out mice for the serotonin transporter (SERT) showed that this interaction was based on extracellular 5-HT availability (Fox et al., 2010).

The increase of HTR observed after the injection of WAY is likely due to an increased activation of 5-HT<sub>2A</sub> receptors. 5-HT<sub>2A</sub> is also thought to be capable of altering cortical sensory gating, the capability of the cortex to adjust its sensitivity to incoming sensory stimuli (Maxwell et al., 2004). It is therefore probable that the mechanism underlying the return to vehicle-like levels of the frontal delta activity, which we argued was context-sensitive, is largely mediated by 5-HT<sub>2A</sub> activation. Indeed, the injection of WAY induced a sustained increase of frontal delta activity as opposed to a shorter lasting change as seen in the occipital delta activity or occipital theta decrease. 5-HT<sub>2A</sub> receptors might have been able either to suppress cortical sensory gating or inhibited the mechanism responsible for the shorter-lasting increase of frontal delta-activity during wake altogether.

Furthermore, the 5-HT<sub>1A</sub> receptors have been shown to reduce neuronal excitability. At the level of the thalamus, such an inhibition of thalamo-cortical neurons should impair the generation of slow waves, as it has been shown that an

activation of these neurons can entrains EEG slow waves (David et al., 2013). Thus, an overactivation of the excitatory 5-HT<sub>2A</sub> receptor occurring with WAY might further activate thalamo-cortical neurons and enhance slow wave activity in the cortex. However, this hypothesis remains to be investigated, especially as David et al. (2013) suggests that thalamo-cortical neurons effectively worked for a narrow frequency range between 0.75 and 1.5 Hz, which is below the rate of the slow waves reported here.

While this model tentatively integrates and addresses most results reported in this thesis, it fails to explain properly the origin of the increased slow wave activity during wake, or the increased sleep latency and alteration of PS homeostasis. However, the only receptor directly studied in this thesis is the 5-HT<sub>1A</sub> receptor, and the proposed effect of 5-HT<sub>2A</sub>-mediated neurotransmission on cortico-cortical signal modulation and processing is pure conjecture and needs to be tested directly.

5-MeO-DMT is known to have a relatively high affinity for other 5-HT-class receptors as well, such as the 5-HT<sub>7</sub> receptors. The 5-HT<sub>7</sub> receptors are known to be involved in the regulation of sleep and circadian rhythmicity. For example, 5-HT<sub>7</sub> knock-out mice or mice injected with a 5-HT<sub>7</sub> selective antagonist showed an increase PS latency and less frequent episodes of PS overall (Hagan et al., 2000; Hedlund et al., 2005). The activation of 5-HT<sub>7</sub> receptor by 5-MeO-DMT should logically reduce PS latency, but this is not what was observed here. However, as 5-HT<sub>1A</sub> is known to regulate the activity and expression of other 5-HT receptors, it is plausible that the injection of 5-MeO-DMT has induced changes in the expression of other 5-HT receptors, such as slowly increasing the expression and concentration of 5-HT<sub>7</sub> receptors leading to the potential increase in PS expression at the end of the 24-hour recording period.

Furthermore, it has been shown that the activation of 5-HT<sub>7</sub> is involved in light-induced phase-shift of the running wheel activity of mice (Shelton et al., 2014). These results suggest that the delay observed in SWS and PS without major rebound could have a circadian origin. Other studies have also demonstrated

that an activation of 5-HT<sub>1A</sub> in the SCN was capable of disrupting light-driven c-fos activity in SCN neurons, further suggesting a potential alteration of the circadian rhythm in 5-MeO-DMT injected-mice, and particularly when injections are conducted at light onset (Glass et al., 1994).

More studies are therefore needed. While using selective antagonists to investigate which receptors are implicated in the alteration of a particular behaviour might help understand better how 5-MeO-DMT acutely affects the brain, 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors, as G-protein coupled receptors, are known to have a wide range of downstream effects. Furthermore, 5-MeO-DMT is a non-specific 5-HT receptor agonist, so it is highly unlikely that one or all behaviours resulting from an injection of 5-MeO-DMT can be traced back to the activation of one receptor.

## 7.3 Exploring the delay of paradoxical sleep

It was reported in Chapter 3 that 5-MeO-DMT injection increased sleep-onset latency, and further increases PS onset. In Chapter 6, after an injection of 5-MeO-DMT following sleep deprivation, while sleep latency was decreased, PS onset occurred after the same time spent in SWS as after the injection of 5-MeO-DMT alone. All of the other experiments presented in this thesis beside Chapter 3 and sleep deprivation in Chapter 6 had important confounding factors, which might be expected to affect animal behaviour or levels of arousal, such as auditory stimulation or manipulation of the animals after one hour of recording to detach either the oculometre or the intracortical-injector. It was, however, apparent that after the injection of WAY, the mice expressed electrophysiological markers of SWS notably sooner than without WAY, suggesting that the latency to sleep onset could be mediated by 5-HT<sub>1A</sub> activation.

The increased latency to PS onset, however, might not be purely pharmacological. Indeed, should a direct activation of a receptor by 5-MeO-DMT cause a delay in PS, then it would be expected that PS onset would occur regardless of the time spent in SWS. The occurrence of PS would therefore always be proportional to

the half-life of 5-MeO-DMT of 12-19 minutes in mice (Ermakova et al., 2022). If PS onset latency was purely pharmacological, then, following a period of sleep deprivation, the time spent in SWS before PS would be more elevated following an injection of 5-MeO-DMT, than without sleep deprivation. It would also be likely that a rebound in PS would be observed. However, PS occurred at the same time after SWS onset following an injection of 5-MeO-DMT with or without sleep deprivation, and still later than in the vehicle condition, and no clear rebound was measured. We hypothesised that 5-MeO-DMT might therefore have influenced sleep states by decreasing PS pressure.

The nature of a 5-MeO-DMT induced decrease in PS pressure remains unknown. Physiologically, such a decrease implies that the putative function of PS has been fulfilled in some other aspect of sleep or wake. PS in animals is characterised by a prominent theta activity in the occipital cortical areas, muscle atonia, and rapid eye movement (Dement, 1958; M. Jouvet et al., 1959c). The acute effect of 5-MeO-DMT injection is a suppression of occipital theta in awake, behaving animals, with no sign of rapid eye movements. Should indeed 5-MeO-DMT functionally replace some aspect of PS, then the function of PS is not nested within these hallmark features.

PS is thought to be primarily involved with emotional memory processing, as numerous reports exist showing a link between altered PS and emotional distress (Deliens et al., 2014). However, other studies suggest that emotional memory processing is not just confined to PS and can still occur during SWS (Cabrera et al., 2024; Deliens et al., 2014). Indeed, successful fear-extinction paradigms in humans were conducted during SWS (Hauner et al., 2013). Furthermore, more PS has not been associated with a stronger emotional decontextualisation of memories (Deliens et al., 2013).

It can be argued however that PS is not responsible for any emotional decontextualisation but might be more involved than SWS when it comes to meaningful, core memories (Deliens et al., 2014). Indeed, human data suggest that memory and associated experienced emotions might strengthen using the memory as a core while

amygdala activity and emotion rising from limbic networks are weakened during PS (Walker & van der Helm, 2009). If emotional memory processing is one of the primary functions of PS, then any action taken towards the processing of emotions and associated memories occurring outside of PS might participate in lowering PS pressure.

Psychedelics are known to profoundly alter wake experience in humans, and especially its emotional aspects (Griffiths et al., 2006; Komater et al., 2012; Osmond, 1957; Vollenweider & Preller, 2020). It has been shown that psilocybin acutely facilitates fear extinction in mice by promoting hippocampal plasticity (Du et al., 2023). Psilocybin and other psychedelics are thought to be involved with the regulation of emotional memory processes. Indeed, the basis for psychedelic-assisted therapy and, therefore, the basis for the therapeutic properties of psychedelics, is that core memories can be altered and more easily decontextualised from stronger emotions by an elevated cerebral plasticity and the reopening of critical periods (Guttman, 1936; Nardou et al., 2023; Rucker et al., 2018). This decontextualisation of emotional memories echoes the one thought to happen during PS. Should that be the case, then the increased PS latency observed after administration of psychedelics in humans and rodents might result from a form of fulfilment of some hypothesised function of PS, thus lowering PS pressure. In our results, we observe a suppression of the theta rhythm which originates from the medial septal nucleus and the hippocampus, as presented in Chapter 1 Subsection 1.3.1, thus supporting the idea that 5-MeO-DMT might have disrupted hippocampal neurocircuitry and could have impacted emotional memory processes.

We also report a slow, cumulative, increase in time spent in PS over 24 hours. This thesis solely focussed on the acute effects of 5-MeO-DMT on sleep and behaviour, and further analysis of recovery days were not performed. It is possible that the injection of 5-MeO-DMT altered the sleep/wake cycle and redistribution of states over a longer period of time. Such observations could provide another insight into the

therapeutic properties of psychedelics. Indeed, as reviewed in Chapter 1, subsection 1.4.1, sleep is affected in patients suffering for major depressive disorders (Wichniak et al., 2017) (Wichniak et al., 2017). As a single administration of psychedelic is a promising therapeutic approach against these illnesses (Rucker et al., 2018), the possibility remains that an acute injection of psychedelics might be sufficient to trigger long-term alterations of the sleep/wake state repartition which in turn might be beneficial for mental health.

## **7.4 Slow waves as a mechanism supporting sleep and therapeutic processes**

### **7.4.1 Slow wave activity and the recovery processes of sleep**

The most notable feature of the EEG activity of mice following the injection of 5-MeO-DMT is the occurrence of global slow waves with neuronal off periods during behavioural wakefulness. Putative regulatory mechanisms were explored in Chapter 6 and a model detailed earlier in this chapter. Chapter 1 and Chapter 3 discussed slow waves as a hallmark electrophysiological feature of sleep, and how the observation of global slow waves calls for the reconsideration of the causal link between slow waves, consciousness, and sleep depth.

Previously, slow wave activity and the incidence of high amplitude slow waves has been correlated with increased sleep pressure (Borbély, 1982; Borbély et al., 2016; Vyazovskiy et al., 2009a, 2011a). However, the presented work suggests that the intrusion of local and global slow-wave events during wake in humans and rodents might, in addition, be a relevant marker of disconnected networks rather than sleep state. There are important examples when slow waves are observed outside of the sleep context: the administration of anaesthetics like ketamine which is known to produce slow waves (Miyasaka & Domino, 1968), and slow waves are common in comatose patients (Husain, 2006). Increased slow wave activity during wakefulness has been associated with lapses in memory processing and attentional processes (Andrillon et al., 2021). Furthermore, slow waves have been proposed

early on to be the default operational mode of a disconnected cortical network, *i.e.* a network not receiving any external input (M. Jouvet & Michel, 1959). *In vitro* studies on dissociated cortical cell cultures show that they exhibit a slow, bistable state akin to slow waves (Hinard et al., 2012). Thus, slow waves as a phenomenon are not confined to sleep, but rather a default state of brain networks.

The results of Chapter 3 and the sleep deprivation experiment detailed in Chapter 6 support this claim. 5-MeO-DMT induces slow waves during an altered wake state. Interestingly, these slow waves did not increase further after sleep deprivation, suggesting that slow waves induced by 5-MeO-DMT are not homeostatically regulated and thus at least partially independent from sleep state.

It is entirely possible that the slow waves reported here are of a different nature than true sleep slow waves. 5-MeO-DMT-induced slow waves exhibit concomitant neuronal off-periods similar to the ones observed at the peak of true sleep slow waves (Nir et al., 2011; Poulet & Petersen, 2008; Vyazovskiy & Harris, 2013). Furthermore, 5-MeO-DMT-induced slow waves occur in a different background, sleep and low arousal state for true-sleep slow wave, wake and elevated arousal for 5-MeO-DMT slow waves. However, we found no major difference between true-sleep and 5-MeO-DMT slow waves.

The possibility that slow-waves can be further subdivided into different types has recently been explored (Bernardi et al., 2019). In humans, the authors have identified two potential subtypes based on their localisation and shape. Frontal-central delta waves are sharper, sawtooth-like, with a concomitant activity in the gamma frequency range, while medial-occipital delta waves are larger, slower waves correlated with a suppression of activity in the gamma frequency range (Bernardi et al., 2019). The wake-like fast activity still present in the EEG recordings following 5-MeO-DMT injection and the wave shape were more similar to frontal-central delta waves. There might be more subtypes to this phenomenon as frontal 5-MeO-DMT waves and occipital ones have been reported to have different regulatory mechanisms. However, the possibility that this results from a different arousal background than



during sleep has not been investigated.

#### 7.4.2 The therapeutic properties of slow waves

Because EEG slow wave activity increases with sleep deprivation, slow waves have been thought to be a leading actor in the recovery effects attributed to sleep. For example, both *in vitro* and *in vivo* recordings suggest that slow waves promote selective synaptic weakening (Bartram et al., 2017; González-Rueda et al., 2018). These changes support the synaptic homeostasis hypothesis (SHY) which posits that during wake, a net increase in synaptic weight is detrimental to further long-term learning due to decreased signal to noise ratio (Tononi & Cirelli, 2003, 2006).

However, sleep-associated recovery processes go beyond synaptic renormalisation. Indeed, restoration of metabolic processes in mice has been associated with sleep homeostasis. On one hand, the decrease of adenosine levels observed during sleep is direct evidence of cellular energy restoration (Porkka-Heiskanen & Kalinchuk, 2011). On the other hand, the restorative properties of sleep can also rise from the clearance of neurotoxic metabolites from the cerebrospinal fluid (Xie et al., 2013). The recovery processes occurring during sleep, therefore, allow a global maintenance of the network. These positive outcomes have been linked to sleep homeostasis (Porkka-Heiskanen & Kalinchuk, 2011; Tononi & Cirelli, 2003, 2006; Xie et al., 2013). Thus, we can theorise that the occurrence of slow waves might induce the structural and/or synaptic changes responsible for the sleep-associated recovery processes.

The observations that increased slow wave activity may lead to functional changes in the brain and therefore represent a potential physiological mechanism of network recovery, has important clinical relevance. Sleep and slow-wave activity are markedly affected in depression, but on the other hand sleep deprivation may have antidepressant properties, albeit short-lasting. Research in humans successfully showed a reduction of depressive-like symptoms with injection of the NMDA-receptor

antagonist ketamine (E. E. Lee et al., 2015). Further studies on humans showed that a single intravenous dose of ketamine acutely induced antidepressant effects within 2 hours and for a week (Zarate et al., 2012). It was proposed that the antidepressant effect of ketamine is mediated by increased slow wave activity (W. C. Duncan et al., 2019; Duncan et al., 2013). Interestingly, the ketamine-induced increase of slow wave activity can be correlated with the levels of brain-derived neurotrophic factors (BDNF) in the hippocampus and neocortex, suggesting elevated plasticity in these areas following an injection of ketamine (Duncan et al., 2013).

Elevated slow wave activity during wakefulness could be responsible for the antidepressant effect observed with classical psychedelics too. A model of the outcome of psychedelic administration in treatment resistant depression suggests that context and the psychedelic experience itself can influence the positive outcome (Carhart-Harris et al., 2018b). Indeed, in humans, it is argued that a “mystical type” experience<sup>1</sup> often correlates with an increased sensation of well-being (Carhart-Harris et al., 2018b). Still in humans, a medium dose of 5-7 mg of 5-MeO-DMT has been shown to induce a more mystical experience than 20 mg of psilocybin, according to the mystical Experience Questionnaire Barsuglia et al. (2018). Furthermore, unpublished data from the Carhart-Harris and Nutt groups from Imperial College London also showed that an elevated dose of psilocybin tends to increase activity in the low frequency range while also being correlated with a more elevated rate of “mystical type” experience (Griffiths et al., 2011). Taken together, these results suggest that increased slow wave activity by administration of psychedelics might be associated with an increased “mystical type” experience in humans and a positive outcome on mental health. While this further supports the notion of slow waves as a key mechanism for the antidepressant action of psychedelics, it also hints at

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<sup>1</sup>A mystical experience can be assessed with the Mystical Experience Questionnaire in humans. The participant is asked to answer on a 0 – 5 scale, where 0 = non/not at all and 5 = extreme (more than ever before in my life and stronger than 4). Questions are divided into four subcategories. Mystical (e.g. “Feeling that you experienced eternity or infinity”), Positive mood (e.g. “Feeling of peace and tranquillity”), Space/Time (e.g. “Sense of being ‘outside of’ time, beyond past and future”) and Ineffability (e.g. “Sense that the experience cannot be described adequately in words”). A participant has had a complete mystical experience when 60% or more of the maximum possible score was endorsed on all subscales. Validation of this questionnaire has been established in Barrett et al., 2015.

slow waves being the source of a higher sense of disconnection during more intense psychedelic experience in humans.

## 7.5 A brain-wide state for psychedelic therapeutic action

In previous parts of the thesis, functional replacement of PS and acute increases in slow wave activity were both presented as possible elements supporting the potential therapeutic effects of classical psychedelics such as 5-MeO-DMT. The functional replacement of PS in therapy has already been explored in SSRI studies. Indeed, PS is heavily dysregulated in depressive disorders (Palagini et al., 2013). While it is thought that most classical antidepressants such as SSRIs and MAOIs initially increase PS latency by suppressing PS expression, studies in rodents involving PS-restriction paradigms showed that the SSRI paroxetine was capable of suppressing PS without the occurrence of a subsequent rebound (McCarthy et al., 2016). The authors argued that SSRIs suppress the expression of classical electrophysiological markers of PS such as the hippocampal theta and muscle atonia, thus protecting a hidden function of PS which in turn prevents a rebound of PS. This supports the notion that the function of PS is not bound to current hallmark electrophysiological features of PS.

However, should antidepressants functionally replace PS, this does not explain why the same rebound in PS was observed after PS-deprivation and PS-deprivation with antidepressants. Should antidepressant action exert the same function as PS, then the rebound following PS-deprivation with antidepressants should be lower than with sleep deprivation alone. Therefore, a suppression of the electrophysiological markers of PS might affect some but not all of PS-associated functions. While the execution of some functions of PS is possible with antidepressants, and by extension psychedelics, it is highly probable that they can only supplant some aspect of PS and not all.

Slow waves, or the lack thereof, have also been associated with acute but short-lasting antidepressant action. Indeed, early studies of sleep restriction protocols showed that sleep deprivation can have a short-term therapeutic effect in patients with depression (Pflug & Tolle, 1971). This suggests that the lack of sleep could be helpful to patients with depression. However, several other studies also point out that this antidepressant effect is reversed as soon as the patient goes to sleep (Boland et al., 2017). The recovery processes of sleep in these studies might therefore be detrimental to patients with depression. Indeed, while sleep might be able to restore brain networks towards a given baseline level driven by homeostatic regulation of sleep states (and therefore by slow waves), should that baseline be equivalent to a depressive state, the occurrence of slow waves would be detrimental and not therapeutic.

Our findings point towards the occurrence of a paradoxical altered state of vigilance. Indeed, acute injections of 5-MeO-DMT produced slow waves during wakefulness, which we propose might be the basis of emotional memory processing phenomenon. It is possible that other psychedelics might exert their therapeutic effects through processes relying on elevated slow wave activity as well. However, we think that for these slow waves to have such a therapeutic effect requires their global occurrence during the wake state.

It was argued in subsection 7.4.2 that slow wave recovery processes might be related to synaptic plasticity processes. We therefore infer that during sleep, the synaptic plasticity restores brain activity towards a hypothetical endogenous baseline, resulting in the synaptic downscaling reported in previous studies. However, the occurrence of such processes during wake opens the possibility of exogenous elements such as cues from the environment or therapy to disrupt such recovery processes. Thus, a speculative thought would be that the occurrence of global slow waves is likely to correspond to a highly and context-dependent plastic, default mode of the cortical network. The origin of disconnection associated with it however

remains to be investigated.

## 7.6 Conclusion

The work presented in this thesis showed that the administration of 5-MeO-DMT can acutely alter behaviour, brain activity, sleep architecture and arousal in mice. The disruption of brain activity results in the occurrence of an altered wake state with global slow waves. These slow waves, while first described as "sleep-like" do not seem to be regulated by sleep processes, nor do they impact subsequent episodes of slow wave sleep, but rather might be modulated by cortical processes relying on elements of wake such as an elevated arousal and behavioural responses to external stimuli. These observations, together with the notion that slow waves possess recovery properties, suggest that the occurrence of sleep-processes such as slow waves during wake might be responsible for the therapeutic properties of psychedelics. Further studies are needed to better characterise the generation and regulation of these processes during wake as well as the direction of their synaptic changes, and to investigate the alteration of PS homeostasis induced by psychedelics.



# Appendices





# A

## Analysis tables of Chapter 3

**Table A.1:** Statistical analyses for figure 3.3

| Variables                                                                                                      | Simple Linear Regression                                                                                                              | Line difference                                              |
|----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Head twitch count<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Drug | <b>5-MeO-DMT</b><br>Slope = $-0.28 \pm 0.064$<br>$R^2 = 0.83$<br><br><i>Line different from zero</i><br>$F_{14} = 19.43$ ; $p < *$    | <b>Vehicle vs 5-MeO-DMT</b><br>$F_{18} = 0.18.52$ ; $p < **$ |
|                                                                                                                | <b>Vehicle</b><br>$R^2 = 0.017$<br>Slope = $-0.0023 \pm 0.01$<br><br><i>Line different from zero</i><br>$F_{14} = 0.070$ ; $p = 0.80$ |                                                              |

| Variables                                                                      | Paired t test                                         |
|--------------------------------------------------------------------------------|-------------------------------------------------------|
| <b>Dependant Variable 1:</b> Head twitch count<br><br><b>WS factor 1:</b> Drug | <b>Vehicle vs 5-MeO-DMT</b><br>$t_3 = 3.96$ ; $p < *$ |

**Table A.2:** Statistical analyses for figure 3.4

| Variables                                                                                                                | One-way ANOVA with repeated measures                        | Multiple comparison (Tukey HSD)              |
|--------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|----------------------------------------------|
| <b>Dependant Variable 1:</b> Latency from bowl placement to bowl interaction<br><br><b>WS factor 1:</b> Condition        | Effect of <b>Condition</b><br>$F_{2,6} = 7.07$ ; $p < *$    | Baseline = a<br>Vehicle = a<br>5-MeO-DMT = b |
| <b>Dependant Variable 1:</b> Latency from bowl placement to pellet eating behaviour<br><br><b>WS factor 1:</b> Condition | Effect of <b>Condition</b><br>$F_{2,6} = 28.95$ ; $p < ***$ | Baseline = a<br>Vehicle = a<br>5-MeO-DMT = b |

| Variables                                                                                             | One-way ANOVA with repeated measures                         |
|-------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Amount of running episode<br><br><b>WS factor 1:</b> Condition           | Effect of <b>Condition</b><br>$F_{2,6} = 2.854$ ; $p = 0.13$ |
| <b>Dependant Variable 1:</b> Average duration of running episode<br><br><b>WS factor 1:</b> Condition | Effect of <b>Condition</b><br>$F_{2,6} = 2.169$ ; $p = 0.20$ |
| <b>Dependant Variable 1:</b> Average running speed<br><br><b>WS factor 1:</b> Condition               | Effect of <b>Condition</b><br>$F_{2,6} = 2.81$ ; $p = 0.14$  |
| <b>Dependant Variable 1:</b> Maximum running speed<br><br><b>WS factor 1:</b> Condition               | Effect of <b>Condition</b><br>$F_{2,6} = 1.81$ ; $p = 0.24$  |

| Variables                                                                            | Friedman test                                               |
|--------------------------------------------------------------------------------------|-------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Total distance ran<br><br><b>WS factor 1:</b> Condition | Effect of <b>Condition</b><br>$F_{2,6} = 4.50$ ; $p = 0.12$ |

**Table A.3:** Statistical analyses for figure 3.6.A-D

| Variables                                                                                                                                                                                                                               | Mixed-effects analysis                                                                                                                                                                                         | Multiple comparison (Fisher's LSD)                                                                                                                                                                                                  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Log transformed frontal EEG power density for each frequency bins (Freq = 0.5:0.25:20) between 0 - 30 minutes post injection.<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition    | <b>Effect of Frequency</b><br>$F_{76,684} = 277.5; p < ****$<br><br><b>Effect of Condition</b><br>$F_{2,18} = 13.32; p < ***$<br><br><b>Effect of Frequency x Condition</b><br>$F_{152,1060} = 4.25; p < ****$ | <b>Baseline vs Vehicle</b><br>[7.75:10]<br><br><b>Baseline vs 5-MeO-DMT</b><br>[0.75:7.75]; [12.5:12.75];<br>13.5; [14:17.75];<br>[18.25:19.5]<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[0.75:6.75]; [8.5:8.75];<br>11.75; [12.5:19.5] |
| <b>Dependant Variable 1:</b> Log transformed occipital EEG power density for each frequency bins (Freq = 0.5:0.25:20) between 0 - 30 minutes post injection.<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition  | <b>Effect of Frequency</b><br>$F_{76,608} = 330.5; p < ****$<br><br><b>Effect of Condition</b><br>$F_{2,16} = 1.70; p = 0.21$<br><br><b>Effect of Frequency x Condition</b><br>$F_{152,908} = 14.15; p < ****$ | <b>Baseline vs Vehicle</b><br>[8.25:9.25]; 9.75<br><br><b>Baseline vs 5-MeO-DMT</b><br>1; [2.5:2.5]; [6.25:12]<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[0.25:5.5]; [6.75:11.75]                                                       |
| <b>Dependant Variable 1:</b> Log transformed frontal EEG power density for each frequency bins (Freq = 0.5:0.25:20) between 60 - 120 minutes post injection<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition   | <b>Effect of Frequency</b><br>$F_{77,693} = 208.4; p < ****$<br><br><b>Effect of Condition</b><br>$F_{2,18} = 0.21; p = 0.82$<br><br><b>Effect of Frequency x Condition</b><br>$F_{154,1074} = 3.29; p < ****$ | <b>Baseline vs Vehicle</b><br>$p > 0.05$<br><br><b>Baseline vs 5-MeO-DMT</b><br>$p > 0.05$<br><br><b>Vehicle vs 5-MeO-DMT</b><br>$p > 0.05$                                                                                         |
| <b>Dependant Variable 1:</b> Log transformed occipital EEG power density for each frequency bins (Freq = 0.5:0.25:20) between 60 - 120 minutes post injection<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition | <b>Effect of Frequency</b><br>$F_{76,608} = 186.2; p < ****$<br><br><b>Effect of Condition</b><br>$F_{2,16} = 0.57; p = 0.58$<br><br><b>Effect of Frequency x Condition</b><br>$F_{152,908} = 3.41; p < ****$  | <b>Baseline vs Vehicle</b><br>[0.75:1]; [1.75:2]; [8.75:10];<br>16.25<br><br><b>Baseline vs 5-MeO-DMT</b><br>0.75; [3:4]; [8.5:10.75];<br>11.25; 13.25; 19.5<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[3:3.5]                          |

**Table A.4:** Statistical analyses for figure 3.6.E-G

| Variables                                                                                                                                                                                    | Mixed-effects analysis                                                                                                                                                                             | Multiple comparison<br>(Fisher's LSD)                                                                                                       |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Average area under curve of log transformed frontal EEG power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition   | Effect of <b>Time</b><br>$F_{24,216} = 1.54; p = 0.057$<br><br>Effect of <b>Condition</b><br>$F_{2,18} = 11.42; p < ***$<br><br>Effect of <b>Time x Condition</b><br>$F_{48,65} = 1.45; p = 0.081$ | <b>Baseline vs Vehicle</b><br>$p > 0.05$<br><br><b>Baseline vs 5-MeO-DMT</b><br>20; [35; 40]<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[10:40]  |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed Occipital EEG power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition | Effect of <b>Time</b><br>$F_{24,192} = 1.64; p < *$<br><br>Effect of <b>Condition</b><br>$F_{2,16} = 15.41; p < ***$<br><br>Effect of <b>Time x Condition</b><br>$F_{48,60} = 1.33; p = 0.14$      | <b>Baseline vs Vehicle</b><br>5<br><br><b>Baseline vs 5-MeO-DMT</b><br>20; [30:50]<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[5:35]; 45         |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed Occipital EEG power density between 6 - 10 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition  | Effect of <b>Time</b><br>$F_{24,192} = 0.87; p = 0.64$<br><br>Effect of <b>Condition</b><br>$F_{2,16} = 4.07; p < *$<br><br>Effect of <b>Time x Condition</b><br>$F_{48,60} = 3.33; p < ****$      | <b>Baseline vs Vehicle</b><br>100; 120<br><br><b>Baseline vs 5-MeO-DMT</b><br>[5:30]; 120<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[5:45]; 100 |

**Table A.5:** Statistical analyses for figure 3.8.A-C

| Variables                                                                                                                      | ANOVA results<br>or non-parametric                                          | Multiple comparison                                            |
|--------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Latency from injection to first consolidate episode of SWS sleep<br><b>WS factor 1:</b> Condition | <i>Friedman test</i><br>$\chi^2(2) = 7.75; p < *$                           | <i>Dunn's</i><br>Baseline = a<br>Vehicle = ab<br>5-MeO-DMT = b |
| <b>Dependant Variable 1:</b> Latency from injection to first consolidate episode of PS sleep<br><b>WS factor 1:</b> Condition  | <i>One-way ANOVA with repeated measures</i><br>$F_{2,14} = 25.22; p < ****$ | <i>Tukey's</i><br>Baseline = a<br>Vehicle = b<br>5-MeO-DMT = c |
| <b>Dependant Variable 1:</b> Latency from SWS onset to first consolidate episode of PS sleep<br><b>WS factor 1:</b> Condition  | <i>One-way ANOVA with repeated measures</i><br>$F_{2,14} = 7.16; p < **$    | <i>Tukey's</i><br>Baseline = a<br>Vehicle = a<br>5-MeO-DMT = b |

**Table A.6:** Statistical analyses for figure 3.8.D-F

| Variables                                                                                                                   | Mixed-effects analysis                                                                                                                                                                                   | Multiple comparison (Tuckey's)                                                |
|-----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Time spent in W<br><br><b>WS factor 1:</b> Condition<br><br><b>WS factor 2:</b> Light period   | Effect of <b>Condition</b><br>$F_{2,18} = 1.026$ ; $p = 0.40$<br><br>Effect of <b>Light</b><br>$F_{1,9} = 210.4$ ; $p < ****$<br><br>Effect of <b>Condition x Light</b><br>$F_{2,10} = 13.21$ ; $p < **$ | <b>Light</b><br>Baseline = a<br>Vehicle = a<br>5-MeO-DMT = a                  |
|                                                                                                                             |                                                                                                                                                                                                          | <b>Dark</b><br>Baseline = a<br>Vehicle = a<br>5-MeO-DMT = b                   |
|                                                                                                                             |                                                                                                                                                                                                          | <b>Light vs Dark</b><br>Baseline < ****<br>Vehicle < ****<br>5-MeO-DMT < **** |
| <b>Dependant Variable 1:</b> Time spent in SWS<br><br><b>WS factor 1:</b> Condition<br><br><b>WS factor 2:</b> Light period | Effect of <b>Condition</b><br>$F_{2,18} = 0.64$ ; $p = 0.40$<br><br>Effect of <b>Light</b><br>$F_{1,9} = 159.1$ ; $p < ****$<br><br>Effect of <b>Condition x Light</b><br>$F_{2,10} = 8.823$ ; $p < **$  | <b>Light</b><br>Baseline = a<br>Vehicle = a<br>5-MeO-DMT = a                  |
|                                                                                                                             |                                                                                                                                                                                                          | <b>Dark</b><br>Baseline = a<br>Vehicle = ab<br>5-MeO-DMT = b                  |
|                                                                                                                             |                                                                                                                                                                                                          | <b>Light vs Dark</b><br>Baseline < ****<br>Vehicle < ****<br>5-MeO-DMT < **** |
| <b>Dependant Variable 1:</b> Time spent in PS<br><br><b>WS factor 1:</b> Condition<br><br><b>WS factor 2:</b> Light period  | Effect of <b>Condition</b><br>$F_{2,18} = 2.41$ ; $p = 0.12$<br><br>Effect of <b>Light</b><br>$F_{1,9} = 193.9$ ; $p < ****$<br><br>Effect of <b>Condition x Light</b><br>$F_{2,10} = 1.33$ ; $p = 0.31$ | <b>Light</b><br>Baseline = a<br>Vehicle = a<br>5-MeO-DMT = a                  |
|                                                                                                                             |                                                                                                                                                                                                          | <b>Dark</b><br>Baseline = ab<br>Vehicle = a<br>5-MeO-DMT = b                  |
|                                                                                                                             |                                                                                                                                                                                                          | <b>Light vs Dark</b><br>Baseline < ****<br>Vehicle < ****<br>5-MeO-DMT < **** |

**Table A.7:** Statistical analyses for figure 3.8.G-K

| Variables                                                                                                                              | Two-way ANOVA                                                                                                                                                                                                  | Multiple comparison (Tukey's)                                                                                                  |
|----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Percent of sleep time spent in PS<br><br><b>WS factor 1:</b> Condition<br><br><b>WS factor 2:</b> ZT Time | Effect of <b>Condition</b><br>$F_{2,18} = 0.60$ ; $p = 0.40$<br><br>Effect of <b>ZT Time</b><br>$F_{23,207} = 6.57$ ; $p < ****$<br><br>Effect of <b>Condition x ZT Time</b><br>$F_{46,211} = 1.45$ ; $p < *$  | <b>Baseline vs Vehicle</b><br>11<br><br><b>Baseline vs 5-MeO-DMT</b><br>1; 11<br><br><b>Vehicle vs 5-MeO-DMT</b><br>$p > 0.05$ |
| <b>Dependant Variable 1:</b> Amount of brief awakenings<br><br><b>WS factor 1:</b> Condition<br><br><b>WS factor 2:</b> ZT Time        | Effect of <b>Condition</b><br>$F_{2,18} = 6.79$ ; $p < **$<br><br>Effect of <b>ZT Time</b><br>$F_{23,207} = 0.99$ ; $p = 0.48$<br><br>Effect of <b>Condition x ZT Time</b><br>$F_{46,211} = 0.93$ ; $p = 0.60$ | N/A                                                                                                                            |

| Variables                                                                                                                   | Two-way ANOVA                                                                                                                                                                                                                     | Multiple comparison (Tuckey's)                                                                                                                                       |
|-----------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Cumulative time in W<br><br><b>WS factor 1:</b> Condition<br><br><b>WS factor 2:</b> ZT Time   | Effect of <b>Condition</b><br>$F_{2,14} = 0.59$ ; $p = 0.50$ (GG)<br><br>Effect of <b>ZT Time</b><br>$F_{46,322} = 697.1$ ; $p < ****$ (GG)<br><br>Effect of <b>Condition x ZT Time</b><br>$F_{92,644} = 3.70$ ; $p < *$ (GG)     | <b>Baseline vs Vehicle</b><br>0.5<br><br><b>Baseline vs 5-MeO-DMT</b><br>[0.5;7]; [8.5;9]<br><br><b>Vehicle vs 5-MeO-DMT</b><br>1                                    |
| <b>Dependant Variable 1:</b> Cumulative time in SWS<br><br><b>WS factor 1:</b> Condition<br><br><b>WS factor 2:</b> ZT Time | Effect of <b>Condition</b><br>$F_{2,14} = 0.55$ ; $p = 0.50$ (GG)<br><br>Effect of <b>ZT Time</b><br>$F_{46,322} = 957.2$ ; $p < ****$ (GG)<br><br>Effect of <b>Condition x ZT Time</b><br>$F_{92,644} = 2.83$ ; $p = 0.085$ (GG) | N/A                                                                                                                                                                  |
| <b>Dependant Variable 1:</b> Cumulative time in PS<br><br><b>WS factor 1:</b> Condition<br><br><b>WS factor 2:</b> ZT Time  | Effect of <b>Condition</b><br>$F_{2,14} = 0.37$ ; $p = 0.70$ (GG)<br><br>Effect of <b>ZT Time</b><br>$F_{46,322} = 759.2$ ; $p < ****$ (GG)<br><br>Effect of <b>Condition x ZT Time</b><br>$F_{92,644} = 5.90$ ; $p < ****$ (GG)  | <b>Baseline vs Vehicle</b><br>[2; 6]<br><br><b>Baseline vs 5-MeO-DMT</b><br>[1.7]; 15.5; [16.5;24]<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[1.5;2]; [3.5;4]; [14.5;24] |

**Table A.8:** Statistical analyses for figure 3.9

| Variables                                                                                                                                                                                                   | Mixed-effects analysis                                                                                                                                                                                         | Multiple comparison<br>(Fisher's LSD)                                                                                                                                                                         |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Log transformed frontal EEG power density for each frequency bins (Freq = 0.5:0.25:20) during SWS.<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition   | Effect of <b>Frequency</b><br>$F_{77,693} = 591.7; p < ****$<br><br>Effect of <b>Condition</b><br>$F_{2,18} = 0.11; p = 0.90$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{154,1074} = 6.92; p < ****$ | <b>Baseline vs Vehicle</b><br>$p > 0.05$<br><br><b>Baseline vs 5-MeO-DMT 1</b><br><br><b>Vehicle vs 5-MeO-DMT</b><br>$p > 0.05$                                                                               |
| <b>Dependant Variable 1:</b> Log transformed occipital EEG power density for each frequency bins (Freq = 0.5:0.25:20) during SWS.<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition | Effect of <b>Frequency</b><br>$F_{77,616} = 504.9; p < ****$<br><br>Effect of <b>Condition</b><br>$F_{2,16} = 2.59; p = 0.11$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{154,920} = 4.38; p < ****$  | <b>Baseline vs Vehicle</b><br>$[0.75:3.5]; [5:5.25]; 12.25$<br><br><b>Baseline vs 5-MeO-DMT</b><br>$[0.75:3.25]; [5:5.75]$<br><br><b>Vehicle vs 5-MeO-DMT</b><br>$[0.75:3]; 8.25$                             |
| <b>Dependant Variable 1:</b> Log transformed frontal EEG power density for each frequency bins (Freq = 0.5:0.25:20) during PS.<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition    | Effect of <b>Frequency</b><br>$F_{77,693} = 260.1; p < ****$<br><br>Effect of <b>Condition</b><br>$F_{2,18} = 1.90; p = 0.18$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{154,1074} = 1.93; p < ****$ | <b>Baseline vs Vehicle</b><br>$[1.75:2.75]; 3.5; 14$<br><br><b>Baseline vs 5-MeO-DMT</b><br>$[3.25:4.25]; [4.75:5.5]; 6$<br><br><b>Vehicle vs 5-MeO-DMT</b><br>$[1.5:2.75]; [5.25:6.25]; [6.75:7]; 12.75; 14$ |
| <b>Dependant Variable 1:</b> Log transformed occipital EEG power density for each frequency bins (Freq = 0.5:0.25:20) during PS.<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition  | Effect of <b>Frequency</b><br>$F_{77,616} = 472.3; p < ****$<br><br>Effect of <b>Condition</b><br>$F_{2,16} = 0.45; p = 0.65$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{154,920} = 1.34; p < **$    | <b>Baseline vs Vehicle</b><br>$p > 0.05$<br><br><b>Baseline vs 5-MeO-DMT</b><br>$[2.5:2.75]$<br><br><b>Vehicle vs 5-MeO-DMT</b><br>$[1.25:2]; [2.5:2.75]; 4.75; [6:7]; [7.5:7.75]; 8.25; 15.75$               |



# B

Analysis tables of Chapter 4

**Table B.1:** Statistical analyses for figure 4.3

| Variables                                                                                      | One-way ANOVA with repeated measures                        |
|------------------------------------------------------------------------------------------------|-------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Latency to initialisation<br><b>WS factor 1:</b> Condition        | Effect of <b>Condition</b><br>$F_{2,4} = 0.79$ ; $p = 0.47$ |
| <b>Dependant Variable 1:</b> Rate of omission<br><b>WS factor 1:</b> Condition                 | Effect of <b>Condition</b><br>$F_{2,4} = 1.14$ ; $p = 0.35$ |
| <b>Dependant Variable 1:</b> Latency to an incorrect response<br><b>WS factor 1:</b> Condition | Effect of <b>Condition</b><br>$F_{2,4} = 0.88$ ; $p = 0.43$ |
| <b>Dependant Variable 1:</b> Latency to premature response<br><b>WS factor 1:</b> Condition    | Effect of <b>Condition</b><br>$F_{2,4} = 0.90$ ; $p = 0.43$ |

| Variables                                                                                   | Friedman's test                                                | Multiple comparison (Dunn's)                          |
|---------------------------------------------------------------------------------------------|----------------------------------------------------------------|-------------------------------------------------------|
| <b>Dependant Variable 1:</b> Rate of accuracy<br><b>WS factor 1:</b> Condition              | Effect of <b>Condition</b><br>$\chi^2(2) = 5.25$ ; $p = 0.079$ | N/A                                                   |
| <b>Dependant Variable 1:</b> Amount of trials<br><b>WS factor 1:</b> Condition              | Effect of <b>Condition</b><br>$\chi^2(2) = 0.00$ ; $p > 0.99$  | N/A                                                   |
| <b>Dependant Variable 1:</b> Latency to a correct response<br><b>WS factor 1:</b> Condition | Effect of <b>Condition</b><br>$\chi^2(2) = 5.25$ ; $p = 0.079$ | N/A                                                   |
| <b>Dependant Variable 1:</b> Latency to collect the reward<br><b>WS factor 1:</b> Condition | Effect of <b>Condition</b><br>$\chi^2(2) = 6.25$ ; $p < *$     | Baseline = a<br>Control = ab<br>Sleep deprivation = b |

**Table B.2:** Statistical analyses for figure 4.6

| Variables                                   | Mixed-effects analysis                                                 | Multiple comparison (Fisher's LSD)                                          |
|---------------------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Pupil diameter | Effect of <b>Time</b><br>$F_{60,300} = 26.21$ ; $p < ****$             | <b>Baseline vs Vehicle</b><br>0                                             |
| <b>WS factor 1:</b> Time                    | Effect of <b>Condition</b><br>$F_{2,9} = 2.736$ ; $p = 0.11$           | <b>Baseline vs 5-MeO-DMT</b><br>[0:17]; 48; [56:57]; 60                     |
| <b>WS factor 2:</b> Condition               | Effect of <b>Time x Condition</b><br>$F_{120,422} = 9.32$ ; $p < ****$ | <b>Vehicle vs 5-MeO-DMT</b><br>[0:16]; 19; 21; 48; 53;<br>[56:57]; [59:60]; |

**Table B.3:** Statistical analyses for figure 4.7.A-B

| Variables                                                                                                             | Mixed-effects analysis                                                     | Multiple comparison (Fisher's LSD)                  |
|-----------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|-----------------------------------------------------|
| <b>Dependant Variable 1:</b> Log transformed frontal EEG power density for each frequency bins (Freq = 0.5:0.25:20)   | Effect of <b>Frequency</b><br>$F_{78,312} = 262.8$ ; $p < ****$            | <b>Vehicle vs 5-MeO-DMT</b><br>[0.75:4]             |
| <b>WS factor 1:</b> Frequency                                                                                         | Effect of <b>Condition</b><br>$F_{14} = 0.15$ ; $p = 0.72$                 |                                                     |
| <b>WS factor 2:</b> Condition                                                                                         | Effect of <b>Frequency x Condition</b><br>$F_{78,75} = 13.22$ ; $p < ****$ |                                                     |
| <b>Dependant Variable 1:</b> Log transformed occipital EEG power density for each frequency bins (Freq = 0.5:0.25:20) | Effect of <b>Frequency</b><br>$F_{78,234} = 145.1$ ; $p < ****$            | <b>Vehicle vs 5-MeO-DMT</b><br>[0.75:4]; [6.75:8.5] |
| <b>WS factor 1:</b> Frequency                                                                                         | Effect of <b>Condition</b><br>$F_{13} = 0.086$ ; $p = 0.79$                |                                                     |
| <b>WS factor 2:</b> Condition                                                                                         | Effect of <b>Frequency x Condition</b><br>$F_{78,76} = 9.276$ ; $p < ****$ |                                                     |

**Table B.4:** Statistical analyses for figure 4.7.C-F

| Variables                                                                                                                                                                                    | Mixed-effects analysis                                                                                                                                                                                 | Multiple comparison<br>(Fisher's LSD)                     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------|
| <b>Dependant Variable 1:</b> Average area under curve of log transformed frontal EEG power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition   | Effect of <b>Time</b><br>$F_{30,120} = 5.23$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{14} = 2.78$ ; $p = 0.41$<br><br>Effect of <b>Time x Condition</b><br>$F_{30,7} = 5.19$ ; $p < *$    | <b>Vehicle vs 5-MeO-DMT</b><br>[2:12]                     |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed occipital EEG power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition | Effect of <b>Time</b><br>$F_{30,90} = 3.29$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{13} = 4.18$ ; $p = 0.13$<br><br>Effect of <b>Time x Condition</b><br>$F_{30,15} = 2.98$ ; $p < **$   | <b>Vehicle vs 5-MeO-DMT</b><br>[6:14]; 16; 22; 26; 34; 40 |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed occipital EEG power density between 6 - 10 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition  | Effect of <b>Time</b><br>$F_{30,90} = 3.26$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{13} = 8.33$ ; $p = 0.13$<br><br>Effect of <b>Time x Condition</b><br>$F_{30,6} = 5.20$ ; $p < ***$   | <b>Vehicle vs 5-MeO-DMT</b><br>[2:28]; 32; 36; 40; 60     |
| <b>Dependant Variable 1:</b> Pupil diameter<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition                                                                             | Effect of <b>Time</b><br>$F_{40,200} = 7.94$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{15} = 12.28$ ; $p < *$<br><br>Effect of <b>Time x Condition</b><br>$F_{40,121} = 3.43$ ; $p < ****$ | <b>Vehicle vs 5-MeO-DMT</b><br>[0:13]; 19                 |

**Table B.5:** Statistical analyses for figure 4.8

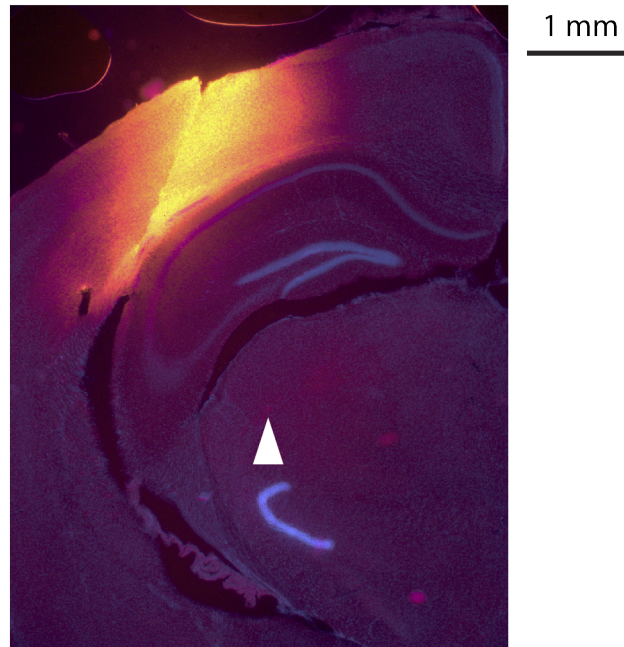
| Variables                                                                                                                                                                                                  | Simple Linear Regression                                                                                          |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Average area under curve of log transformed frontal EEG power density between 0.5 - 4 Hz<br><br><b>Dependant Variable 2:</b> Pupil diameter<br><br><b>WS factor 1:</b> Time   | Slope = $2.17 \pm 0.34$<br>$R^2 = 0.59$<br><br><i>Line different from zero</i><br>$F_{1,29} = 41.29$ ; $p < ****$ |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed occipital EEG power density between 0.5 - 4 Hz<br><br><b>Dependant Variable 2:</b> Pupil diameter<br><br><b>WS factor 1:</b> Time | Slope = $0.62 \pm 0.43$<br>$R^2 = 0.07$<br><br><i>Line different from zero</i><br>$F_{1,29} = 2.08$ ; $p = 0.16$  |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed occipital EEG power density between 6 - 10 Hz<br><br><b>Dependant Variable 2:</b> Pupil diameter<br><br><b>WS factor 1:</b> Time  | Slope = $-0.38 \pm 0.15$<br>$R^2 = 0.18$<br><br><i>Line different from zero</i><br>$F_{1,18} = 6.41$ ; $p < *$    |



# C

## Supplementary materials of Chapter 5

### C.1 Histology



**Figure C.1: Histology results of Chapter 5.** Representative histology picture in an animal implanted intracortically with a 16 channel silicon probe coloured in DiI. Image acquired on a Leica DMR with DFC 500 camera in true colour (red = DiI, blue = DAPI). Brain slice was located at -2.3 antero-posterior. The white arrow points at backlabelled thalamic neurons located in lateral posterior and posterior thalamic nuclei.

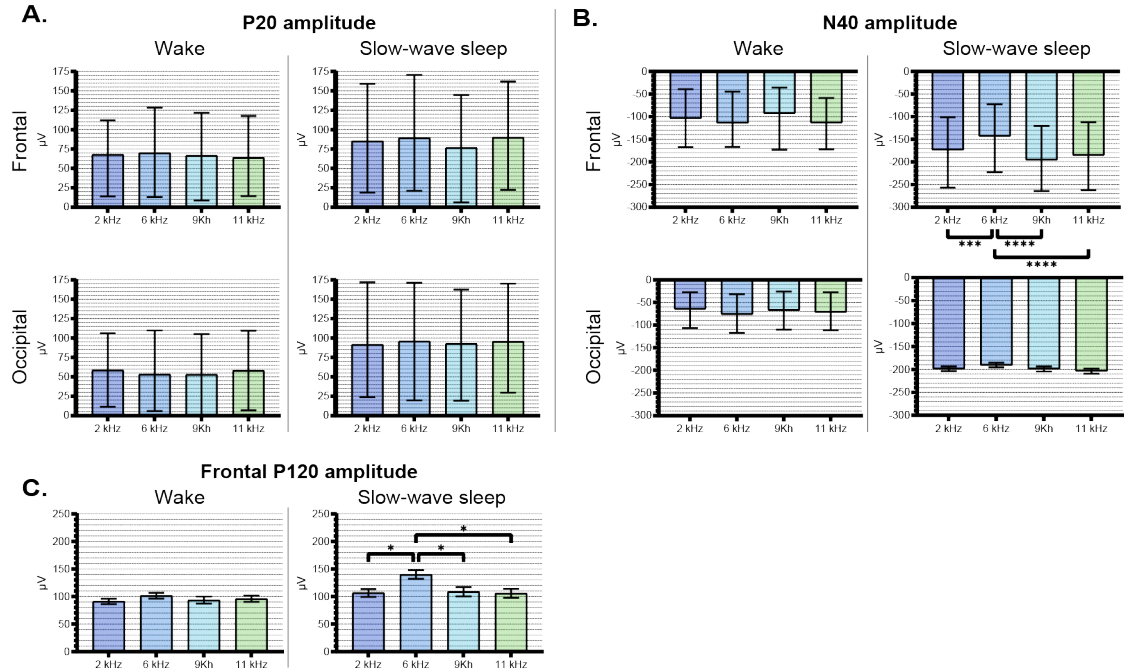
## C.2 ERP component analysis

### C.2.1 Stimulation frequency have little impact on EEG ERP components

In terms of amplitude, it was found that most stimulations, regardless of the frequencies, elicited, on average, the same ERP amplitude, except for 6 kHz which might have decreased the amplitude of the frontal N40 (Kruskal-Wallis, effect of rate  $H_3 = 40.4$ ,  $p < 0.0001$ ; Dunn's multiple comparison  $p < 0.05$  for 6 kHz against all other rates) (Figure 5.B) and increased the frontal P120 during SWS (Welch ANOVA, effect of rate  $W_{3,385} = 4.52$ ,  $p < 0.05$  for 6 kHz against all other rates) (Figure C.2.C).

In terms of latency from auditory stimulation, during wake, it was found that 9 kHz stimulations induced a difference P20 latency compared to 6 kHz stimulations in the frontal derivation (Kruskal-Wallis, effect of rate  $H_3 = 11.29$ ,  $p < 0.05$ ; Dunn's multiple comparison  $p < 0.05$  for 9 kHz against 6 kHz) (Figure C.3.A), and increased N40 latency in the frontal derivation compared to 2kHz and 6 kHz stimulations (Kruskal-Wallis, effect of rate  $H_3 = 10.63$ ,  $p < 0.05$ ; Dunn's multiple comparison  $p < 0.05$  for 9 kHz against 2 and 6 kHz) (Figure C.3.B). During SWS, 6 kHz stimulation elicited seemingly different latencies, with an increase P20 latency in the frontal and (Kruskal-Wallis, effect of rate  $H_3 = 36.54$ ,  $p < 0.0001$ ; Dunn's multiple comparison  $p < 0.05$  for 6 kHz against all other rates) and occipital derivations (Kruskal-Wallis, effect of rate  $H_3 = 31.61$ ,  $p < 0.0001$ ; Dunn's multiple comparison  $p < 0.05$  for 6 kHz against all other rates) (Figure C.3), as well as an increase in N40 latency in the occipital derivation (Kruskal-Wallis, effect of rate  $H_3 = 61.66$ ,  $p < 0.0001$ ; Dunn's multiple comparison  $p < 0.05$  for 6 kHz against all other rates) (Figure C.3.B). During SWS also, the latency of the frontal P120 was increased following a 11 kHz stimulation compared a 6 kHz one (Kruskal-Wallis, effect of rate  $H_3 = 8.40$ ,  $p < 0.05$ ; Dunn's multiple comparison  $p < 0.05$  for 6 kHz against 11 kHz) (Figure C.3.C).

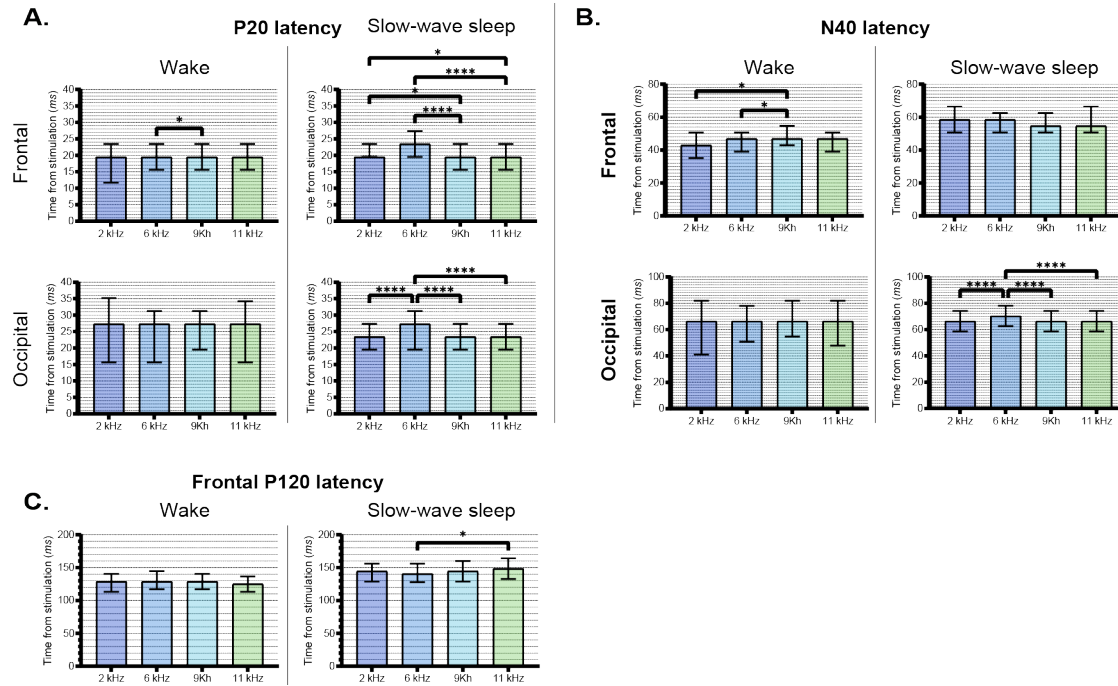




**Figure C.2: EEG ERP components amplitude across wake and sleep. A.** Analysis of P20 across wake (left) and SWS (right) in the frontal (top) and occipital (bottom) EEG derivation. **B.** Analysis of N40 across wake (left) and SWS (right) in the frontal (top) and occipital (bottom) EEG derivation. **C.** Analysis of P120 across wake (left) and SWS (right) in the frontal EEG derivation. There is no occipital visualisation because of the lack of clear P120 component in the occipital derivation during wake and sleep. Data are presented as mean + SEM or median + IQRs for A (all) and B (frontal W + SWS, occipital W only) (\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ ).  $N = 3$  for all panels. See Table C.6 for statistical analyses.

### C.2.2 ERP components change through vigilance states

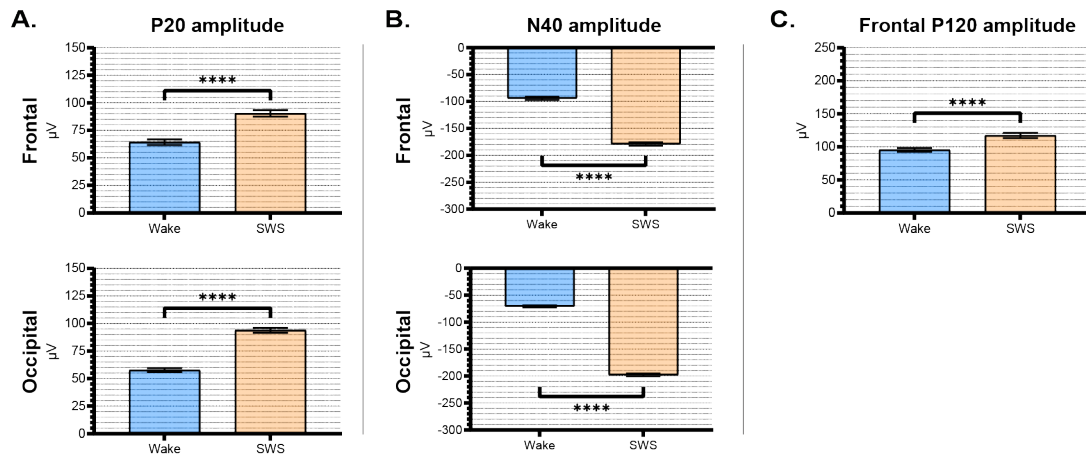
The differences in responses between wake and sleep were then analysed without any distinction between frequencies. It was found that during SWS the amplitude and latency of all components were significantly increased except for a decreased occipital p20 latency. The amplitude of the P20 component was significantly higher during SWS than during wake in the frontal derivation (Welch's  $t$ -test  $t_{2717} = 6.64$ ;  $p < 0.0001$ ) and in the occipital derivation (Welch's  $t$ -test  $t_{4192} = 12.56$ ;  $p = 0.0001$ ) (Figure C.4.A), the amplitude of the N40 component was also increased during SWS in the frontal Welch's  $t$ -test  $t_{2144} = 19.25$ ;  $p < 0.0001$ ) (Figure C.4.B) and occipital derivation (Welch's  $t$ -test  $t_{3165} = 38.87$ ;  $p < 0.0001$ ) (Figure C.4.B), and the amplitude of the P120 was increased in the frontal derivation as well (Welch's



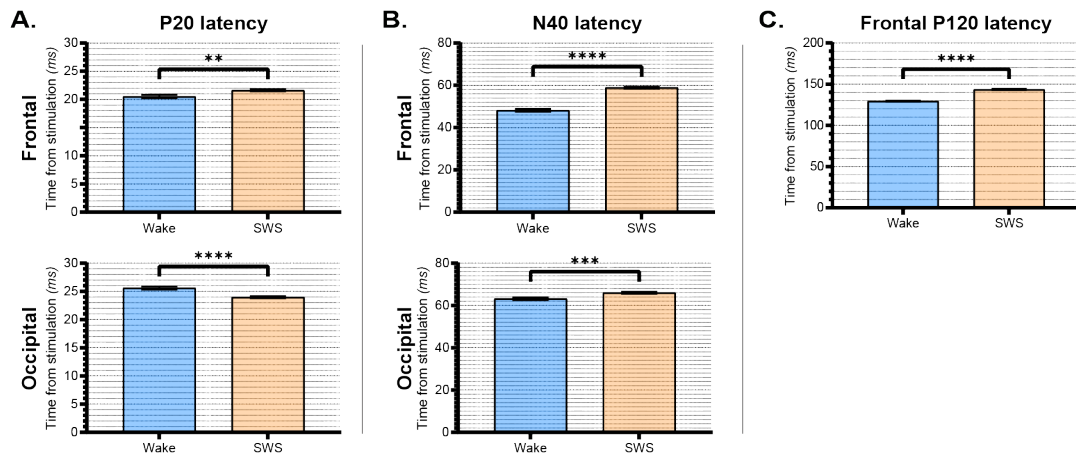
**Figure C.3: EEG ERP components latencies across wake and sleep.** **A.** Analysis of P20 across wake (left) and SWS (right) in the frontal (top) and occipital (bottom) EEG derivation. **B.** Analysis of N40 across wake (left) and SWS (right) in the frontal (top) and occipital (bottom) EEG derivation. **C.** Analysis of P120 across wake (left) and SWS (right) in the frontal EEG derivation. There is no occipital visualisation because of the lack of clear P120 component in the occipital derivation during wake and sleep. Data are presented as median + IQRs (\* $p < 0.05$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ ).  $N = 3$  for all panels. See Table C.7 for statistical analyses.

t-test  $t_{1275} = 4.46$ ;  $p < 0.0001$ ) (Figure C.4.C).

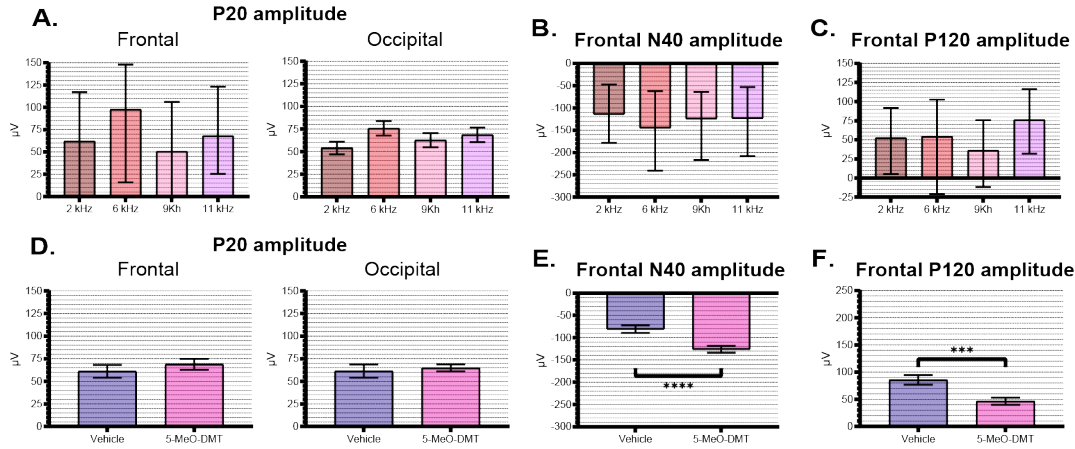
For the latency of each component, P20 occurred at a significantly earlier time during wake than during sleep in the frontal derivation (Welch's t-test  $t_{1883} = 3.06$ ;  $p < 0.01$ ) but at a later time in the occipital derivation (Welch's t-test  $t_{3078} = 4.85$ ;  $p < 0.0001$ ) (Figure C.5.A). N40 latency was increased during SWS in the frontal (Welch's t-test  $t_{1647} = 13.59$ ;  $p < 0.0001$ ) and occipital derivation (Welch's t-test  $t_{2403} = 3.34$ ;  $p < 0.001$ ) (Figure 8.B), and P120 occurred significantly later during SWS than during wake in the frontal derivation (t-test  $t_{1394} = 11.84$ ;  $p < 0.0001$ ) (Figure C.5.C).



**Figure C.4: EEG ERP components amplitude changes between wake and SWS.** **A.** Analysis of P20 in the frontal (top) and occipital (bottom) EEG derivation. **B.** Analysis of P20 in the frontal (top) and occipital (bottom) EEG derivation. **C.** Analysis of P120 in the frontal EEG derivation. There is no occipital visualisation because of the lack of clear P120 component in the occipital derivation during wake and sleep. Data are presented as mean + SEM (\* $p < 0.05$  ; \*\*\* $p < 0.001$  ; \*\*\*\* $p < 0.001$ ).  $N = 3$  for all panels. See Table C.8 for statistical analyses.



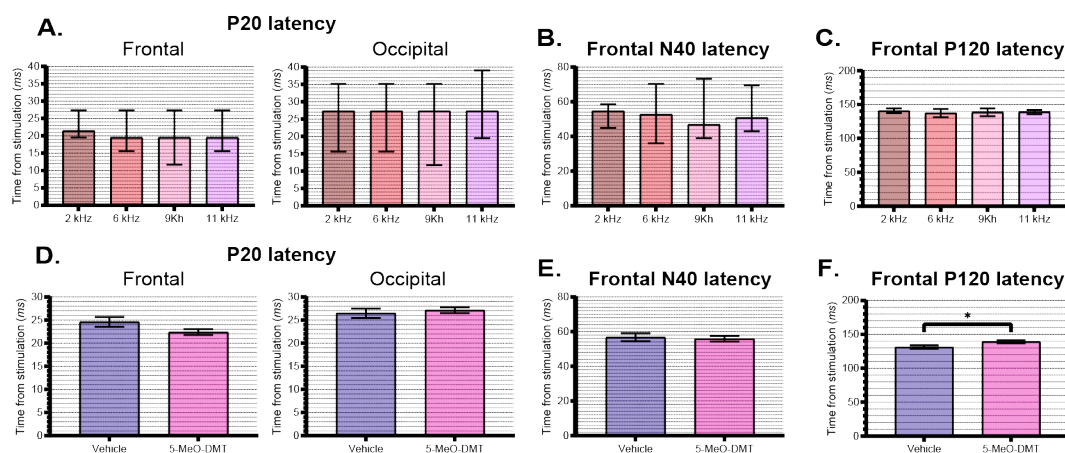
**Figure C.5: EEG ERP components latency changes between wake and SWS.** **A.** Analysis of P20 in the frontal (top) and occipital (bottom) EEG derivation. **B.** Analysis of P20 in the frontal (top) and occipital (bottom) EEG derivation. **C.** Analysis of P120 in the frontal EEG derivation. There is no occipital visualisation because of the lack of clear P120 component in the occipital derivation during wake and sleep. Data are presented as mean + SEM (\*\* $p < 0.01$  ; \*\*\* $p < 0.001$ ).  $N = 3$  for all panels. See Table C.9 for statistical analyses.



**Figure C.6: The effects of 5-MeO-DMT on EEG ERP components amplitude.** **A.** Analysis of P20 across all stimulation frequencies after an injection of 5-MeO-DMT in the frontal (right) and occipital (left) EEG derivation. **B.** Analysis of P20 between 5-MeO-DMT and vehicle in the frontal (right) and occipital (left) EEG derivation. **C.** Analysis of N40 across all stimulation frequencies after an injection of 5-MeO-DMT in the frontal (right) and occipital (left) EEG derivation. **D.** Analysis of P120 across all stimulation frequencies after an injection of 5-MeO-DMT in the frontal (right) and occipital (left) EEG derivation. **E.** Analysis of N40 between 5-MeO-DMT and vehicle in the frontal EEG derivation. **F.** Analysis of P120 between 5-MeO-DMT and vehicle in the frontal EEG derivation. There is no occipital visualisation of N40 and P120 because of the lack of clear N40 and P120 component in the occipital derivation following an injection of 5-MeO-DMT. Data are presented as mean + SEM or median + IQRs for A (frontal), B and C. (\*\*\*) $p < 0.001$ ; (\*\*\*\*) $p < 0.0001$ .  $N = 3$  for all panels. See Table C.10 for statistical analyses.

### C.2.3 ERP components following an injection of 5-MeO-DMT

An analysis of each component of the ERP showed that the different frequencies of stimulation used did not elicit any differences in the amplitudes of the ERPs following an injection of 5-MeO-DMT, with a similar P20 amplitude across different frequencies in the frontal and occipital derivations (Figure C.6.A), and a similar frontal N40 and P120 amplitude across all frequencies (Figure C.6.B-C). Comparing 5-MeO-DMT to vehicle, the injection of either substance elicited the same P20 amplitude in both the frontal and occipital derivations (Figure C.6.D). However, the injection of 5-MeO-DMT induced a deeper N40 (Welch's  $t$ -test  $t_{312.3} = 4.70$ ;  $p < 0.0001$ ) and smaller P120 ( $t$ -test  $t_{224} = 3.63$ ;  $p < 0.001$ ) in the frontal derivation compared to vehicle (Figure C.6.E-F).



**Figure C.7: The effects of 5-MeO-DMT on EEG ERP components latencies.** **A.** Analysis of P20 across all stimulation frequencies after an injection of 5-MeO-DMT in the frontal (right) and occipital (left) EEG derivation. **B.** Analysis of P20 between 5-MeO-DMT and vehicle in the frontal (right) and occipital (left) EEG derivation. **C.** Analysis of N40 across all stimulation frequencies after an injection of 5-MeO-DMT in the frontal (right) and occipital (left) EEG derivation. **D.** Analysis of P120 across all stimulation frequencies after an injection of 5-MeO-DMT in the frontal (right) and occipital (left) EEG derivation. **E.** Analysis of N40 between 5-MeO-DMT and vehicle in the frontal EEG derivation. **F.** Analysis of P120 between 5-MeO-DMT and vehicle in the frontal EEG derivation. There is no occipital visualisation of N40 and P120 because of the lack of clear N40 and P120 component in the occipital derivation following an injection of 5-MeO-DMT. Data are presented as mean + SEM or median + IQRs for A (all) and B (\* $p < 0.0001$ ).  $N = 3$  for all panels. See Table C.11 for statistical analyses.

Regarding the latency of each component, there was no difference in the frontal or occipital derivation regarding the occurrence of P20 (for detailed statistics, please refer to the annexes) (Figure C.7.A), or the frontal N40 and P120 across different stimulation frequencies in the presence of the drug (Figure C.7.B-C). Comparing 5-MeO-DMT to vehicle, 5-MeO-DMT only delayed the occurrence of the frontal P120 ( $t$ -test  $t_{224} = 2.22$ ;  $p < 0.05$ ) (Figure C.7.F) but did not elicit any change in the latencies of the frontal or occipital P20 (Figure C.7.D), or of the frontal N40 (Figure C.7.E).

### C.3 Analysis tables

**Table C.1:** Statistical analyses for figure 5.4

| Variables                                                                               | Paired t-test            |
|-----------------------------------------------------------------------------------------|--------------------------|
| <b>Dependant Variable 1:</b> Latency from injection to first consolidate episode of SWS | $t_2 = 17.40$ ; $p < **$ |
| <b>WS factor 1:</b> Condition                                                           |                          |
| <b>Dependant Variable 1:</b> Latency from injection to first consolidate episode of PS  | $t_2 = 6.96$ ; $p < *$   |
| <b>WS factor 1:</b> Condition                                                           |                          |

| Variables                                                                                                                                                                                                   | 2-WAY ANOVA with repeated measures                                                                                                                                                                                                                     | Multiple comparison (Fisher's LSD) |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| <b>Dependant Variable 1:</b> Frontal EEG activity during W around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Stimulation frequency   | Effect of <b>Time</b><br>$F_{153, 153} = 10.73$ ; $p = 0.19$ (GG)<br><br>Effect of <b>Stimulation frequency</b><br>$F_{3, 3} = 1.91$ ; $p = 0.40$ (GG)<br><br>Effect of <b>Time x Stimulation frequency</b><br>$F_{459, 459} = 1.94$ ; $p = 0.40$ (GG) | N/A                                |
| <b>Dependant Variable 1:</b> Occipital EEG activity during W around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Stimulation frequency | Effect of <b>Time</b><br>$F_{153, 306} = 1.80$ ; $p = 0.29$ (GG)<br><br>Effect of <b>Stimulation frequency</b><br>$F_{3, 6} = 0.17$ ; $p = 0.76$ (GG)<br><br>Effect of <b>Time x Stimulation frequency</b><br>$F_{459, 918} = 1.16$ ; $p = 0.40$ (GG)  | N/A                                |
| <b>Dependant Variable 1:</b> Frontal EEG activity during SWS around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Stimulation frequency | Effect of <b>Time</b><br>$F_{153, 153} = 4.68$ ; $p = 0.27$ (GG)<br><br>Effect of <b>Stimulation frequency</b><br>$F_{3, 3} = 0.77$ ; $p = 0.41$ (GG)<br><br>Effect of <b>Time x Stimulation frequency</b><br>$F_{459, 459} = 1.37$ ; $p = 0.45$ (GG)  | N/A                                |

|                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                             |     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <p><b>Dependant Variable 1:</b> Occipital EEG activity during SWS around an auditory stimulation (Time = -198.219:3.91:400.011)</p> <p><b>WS factor 1:</b> Time</p> <p><b>WS factor 2:</b> Stimulation frequency</p> | <p>Effect of <b>Time</b><br/> <math>F_{\text{E3},306} = 2.68</math>; <math>p = 0.29</math> (GG)</p> <p>Effect of <b>Stimulation frequency</b><br/> <math>F_{3,6} = 0.19</math>; <math>p = 0.76</math> (GG)</p> <p>Effect of <b>Time x Stimulation frequency</b><br/> <math>F_{4159,938} = 0.50</math>; <math>p = 0.40</math> (GG)</p>       | N/A |
| <p><b>Dependant Variable 1:</b> Frontal EEG activity during W and SWS around an auditory stimulation (Time = -198.219:3.91:400.011)</p> <p><b>WS factor 1:</b> Time</p> <p><b>WS factor 2:</b> Vigilance state</p>   | <p>Effect of <b>Time</b><br/> <math>F_{\text{E3},\text{E3}} = 8.41</math>; <math>p = 0.21</math> (GG)</p> <p>Effect of <b>Vigilance state</b><br/> <math>F_{11} = 31.02</math>; <math>p = 0.11</math> (GG)</p> <p>Effect of <b>Time x Vigilance state</b><br/> <math>F_{\text{E3},\text{E3}} = 10.83</math>; <math>p = 0.19</math> (GG)</p> | N/A |
| <p><b>Dependant Variable 1:</b> Occipital EEG activity during W and SWS around an auditory stimulation (Time = -198.219:3.91:400.011)</p> <p><b>WS factor 1:</b> Time</p> <p><b>WS factor 2:</b> Vigilance state</p> | <p>Effect of <b>Time</b><br/> <math>F_{\text{E3},306} = 3.15</math>; <math>p = 0.20</math> (GG)</p> <p>Effect of <b>Vigilance state</b><br/> <math>F_{11} = 5.91</math>; <math>p = 0.14</math> (GG)</p> <p>Effect of <b>Time x Vigilance state</b><br/> <math>F_{\text{E3},306} = 1.81</math>; <math>p = 0.31</math> (GG)</p>               | N/A |



**Table C.2:** Statistical analyses for figure 5.5

| Variables                                                                                                                                                                                       | ANOVA with repeated measures                                                                                                                                                                                                         | Multiple comparison (Fisher's LSD)                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Log transformed frontal EEG power density for each frequency bins (Freq = 0.5:0.25:20)<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition   | <i>Two-way ANOVA</i><br>Effect of <b>Frequency</b><br>$F_{77,77} = 274.7; p < ****$<br><br>Effect of <b>Condition</b><br>$F_{12} = 976.7; p < *$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{77,77} = 1.67; p < *$          | <b>Vehicle vs 5-MeO-DMT</b><br>1; [1.5:6]; 11.25;<br>[11.75:12.5]; [13:13.25];<br>[14:14.25]; 15.75 |
| <b>Dependant Variable 1:</b> Log transformed occipital EEG power density for each frequency bins (Freq = 0.5:0.25:20)<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition | <i>ME analysis</i><br>Effect of <b>Frequency</b><br>$F_{77,154} = 348.6; p < ****$<br><br>Effect of <b>Condition</b><br>$F_{12} = 52.63; p < *$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{77,76} = 21.18; p < ****$       | <b>Vehicle vs 5-MeO-DMT</b><br>[1.5:4.75]; [5.5:11.5];<br>[12:12.5]; 13.75; 16.75;<br>[19.5:19.75]  |
| <b>Dependant Variable 1:</b> Log transformed LFP power density for each frequency bins (Freq = 0.5:0.25:20)<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition           | <i>Two-way ANOVA</i><br>Effect of <b>Frequency</b><br>$F_{77,154} = 508.6; p < ****$<br><br>Effect of <b>Condition</b><br>$F_{12} = 4.01; p = 0.11$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{154,308} = 10.38; p < ****$ | <b>Vehicle vs 5-MeO-DMT</b><br>[0.75:6]; [6.25:9.5]                                                 |



**Table C.3:** Statistical analyses for figure 5.6

| Variables                                                                                                                                                                                                   | 2-WAY ANOVA with repeated measures                                                                                                                                                                                                                 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Frontal EEG activity during W around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Stimulation frequency   | Effect of <b>Time</b><br>$F_{153,153} = 1.93$ ; $p = 0.40$ (GG)<br><br>Effect of <b>Condition</b><br>$F_{11} = 0.19$ ; $p = 0.74$ (GG)<br><br>Effect of <b>Time x Condition</b><br>$F_{153,153} = 1.27$ ; $p = 0.46$ (GG)                          |
| <b>Dependant Variable 1:</b> Occipital EEG activity during W around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Stimulation frequency | Effect of <b>Time</b><br>$F_{153,306} = 1.80$ ; $p = 0.30$ (GG)<br><br>Effect of <b>Stimulation frequency</b><br>$F_{3,6} = 0.98$ ; $p = 0.44$ (GG)<br><br>Effect of <b>Time x Stimulation frequency</b><br>$F_{459,918} = 0.78$ ; $p = 0.52$ (GG) |
| <b>Dependant Variable 1:</b> Frontal EEG activity during W around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> condition               | Effect of <b>Time</b><br>$F_{153,153} = 2.039$ ; $p = 0.39$ (GG)<br><br>Effect of <b>Condition</b><br>$F_{11} = 81.75$ ; $p = 0.07$ (GG)<br><br>Effect of <b>Time x Condition</b><br>$F_{153,153} = 2.47$ ; $p = 0.36$ (GG)                        |
| <b>Dependant Variable 1:</b> Occipital EEG activity during W around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> condition             | Effect of <b>Time</b><br>$F_{153,153} = 1.95$ ; $p = 0.40$ (GG)<br><br>Effect of <b>Condition</b><br>$F_{11} = 11.92$ ; $p = 0.18$ (GG)<br><br>Effect of <b>Time x Condition</b><br>$F_{153,153} = 3.41$ ; $p = 0.32$ (GG)                         |

**Table C.4:** Statistical analyses for figure 5.8

| Variables                                                                                                                                                                                           | 2-WAY ANOVA with repeated measures                                                                                                                                                                                                                |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> LFP activity during W around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Stimulation frequency   | Effect of <b>Time</b><br>$F_{53,306} = 4.91$ ; $p = 0.11$ (GG)<br><br>Effect of <b>Stimulation frequency</b><br>$F_{3,6} = 1.07$ ; $p = 0.42$ (GG)<br><br>Effect of <b>Time x Stimulation frequency</b><br>$F_{459,918} = 1.04$ ; $p = 0.42$ (GG) |
| <b>Dependant Variable 1:</b> LFP activity during SWS around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Stimulation frequency | Effect of <b>Time</b><br>$F_{53,306} = 1.57$ ; $p = 0.33$ (GG)<br><br>Effect of <b>Stimulation frequency</b><br>$F_{3,6} = 0.71$ ; $p = 0.49$ (GG)<br><br>Effect of <b>Time x Stimulation frequency</b><br>$F_{459,918} = 0.95$ ; $p = 0.45$ (GG) |
| <b>Dependant Variable 1:</b> LFP activity around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Vigilance state                  | Effect of <b>Time</b><br>$F_{53,306} = 1.40$ ; $p = 0.32$ (GG)<br><br>Effect of <b>Vigilance state</b><br>$F_{1,1} = 93.70$ ; $p < *$ (GG)<br><br>Effect of <b>Time x Vigilance state</b><br>$F_{53,306} = 2.23$ ; $p = 0.27$ (GG)                |

**Table C.5:** Statistical analyses for figure 5.9

| Variables                                                                                                                                                                                                          | 2-WAY ANOVA with repeated measures                                                                                                                                                                                                                 |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> LFP activity during wake round an auditory stimulation<br>(Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Stimulation frequency             | Effect of <b>Time</b><br>$F_{153,306} = 1.26$ ; $p = 0.38$ (GG)<br><br>Effect of <b>Stimulation frequency</b><br>$F_{3,6} = 0.85$ ; $p = 0.46$ (GG)<br><br>Effect of <b>Time x Stimulation frequency</b><br>$F_{459,918} = 0.74$ ; $p = 0.50$ (GG) |
| <b>Dependant Variable 1:</b> LFP activity during wake around an auditory stimulation following an injection<br>(Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition | Effect of <b>Time</b><br>$F_{153,306} = 2.54$ ; $p = 0.20$ (GG)<br><br>Effect of <b>Condition</b><br>$F_{1,1} = 0.18$ ; $p = 0.71$ (GG)<br><br>Effect of <b>Time x Condition</b><br>$F_{153,306} = 1.04.47$ ; $p = 0.43$ (GG)                      |
| <b>Dependant Variable 1:</b> MUA activity during wake around an auditory stimulation<br>(Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition                        | Effect of <b>Time</b><br>$F_{153,306} = 8.91$ ; $p = 0.06$ (GG)<br><br>Effect of <b>Condition</b><br>$F_{1,2} = 0.20$ ; $p = 0.69$ (GG)<br><br>Effect of <b>Time x Condition</b><br>$F_{153,306} = 1.45$ ; $p = 0.34$ (GG)                         |

Table C.6: Statistical analyses for figure C.2

| Variables                                                                                                              | Comparison                                                                                               | Multiple comparison                                                      |
|------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Amplitude of frontal P20 peak during W<br><b>WS factor 1:</b> Stimulation frequency       | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 0.74$ ; $p = 0.86$            | N/A                                                                      |
| <b>Dependant Variable 1:</b> Amplitude of occipital P20 peak during W<br><b>WS factor 1:</b> Stimulation frequency     | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 1.60$ ; $p = 0.66$            | N/A                                                                      |
| <b>Dependant Variable 1:</b> Amplitude of frontal P20 peak during SWS<br><b>WS factor 1:</b> Stimulation frequency     | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 4.56$ ; $p = 0.21$            | N/A                                                                      |
| <b>Dependant Variable 1:</b> Amplitude of occipital P20 peak during SWS<br><b>WS factor 1:</b> Stimulation frequency   | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 1.84$ ; $p = 0.61$            | N/A                                                                      |
| <b>Dependant Variable 1:</b> Amplitude of frontal N40 trough during W<br><b>WS factor 1:</b> Stimulation frequency     | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 6.52$ ; $p = 0.089$           | N/A                                                                      |
| <b>Dependant Variable 1:</b> Amplitude of occipital N40 trough during W<br><b>WS factor 1:</b> Stimulation frequency   | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 2.97$ ; $p = 0.40$            | N/A                                                                      |
| <b>Dependant Variable 1:</b> Amplitude of frontal N40 trough during SWS<br><b>WS factor 1:</b> Stimulation frequency   | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 40.4$ ; $p < ****$            | <i>Dunn's</i><br>2 kHz = a<br>6 kHz = b<br>9 kHz = a<br>11 kHz = a       |
| <b>Dependant Variable 1:</b> Amplitude of occipital N40 trough during SWS<br><b>WS factor 1:</b> Stimulation frequency | <i>Welch one-way ANOVA</i><br>Effect of <b>Stimulation frequency</b><br>$W_{3,1052} = 1.12$ ; $p = 0.34$ | N/A                                                                      |
| <b>Dependant Variable 1:</b> Amplitude of frontal P120 peak during W<br><b>WS factor 1:</b> Stimulation frequency      | <i>Welch one-way ANOVA</i><br>Effect of <b>Stimulation frequency</b><br>$W_{3,1052} = 0.70$ ; $p = 0.55$ | N/A                                                                      |
| <b>Dependant Variable 1:</b> Amplitude of frontal P120 peak during SWS<br><b>WS factor 1:</b> Stimulation frequency    | <i>Welch one-way ANOVA</i><br>Effect of <b>Stimulation frequency</b><br>$W_{3,385} = 4.52$ ; $p < **$    | <i>Dunnett's T3</i><br>2 kHz = a<br>6 kHz = b<br>9 kHz = a<br>11 kHz = a |

**Table C.7:** Statistical analyses for figure C.3

| Variables                                                                                                            | Kruskal-Wallis                                                     | Multiple comparison (Dunn's)                        |
|----------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|-----------------------------------------------------|
| <b>Dependant Variable 1:</b> Latency of frontal P20 peak during W<br><b>WS factor 1:</b> Stimulation frequency       | Effect of <b>Stimulation frequency</b><br>$H(3) = 11.29; p < *$    | 2 kHz = ab<br>6 kHz = a<br>9 kHz = b<br>11 kHz = ab |
| <b>Dependant Variable 1:</b> Latency of occipital P20 peak during W<br><b>WS factor 1:</b> Stimulation frequency     | Effect of <b>Stimulation frequency</b><br>$H(3) = 0.61; p = 0.67$  | N/A                                                 |
| <b>Dependant Variable 1:</b> Latency of frontal P20 peak during SWS<br><b>WS factor 1:</b> Stimulation frequency     | Effect of <b>Stimulation frequency</b><br>$H(3) = 36.54; p < ****$ | 2 kHz = a<br>6 kHz = a<br>9 kHz = b<br>11 kHz = b   |
| <b>Dependant Variable 1:</b> Latency of occipital P20 peak during SWS<br><b>WS factor 1:</b> Stimulation frequency   | Effect of <b>Stimulation frequency</b><br>$H(3) = 31.61; p < ****$ | 2 kHz = a<br>6 kHz = b<br>9 kHz = a<br>11 kHz = a   |
| <b>Dependant Variable 1:</b> Latency of frontal N40 trough during W<br><b>WS factor 1:</b> Stimulation frequency     | Effect of <b>Stimulation frequency</b><br>$H(3) = 10.63; p < *$    | 2 kHz = a<br>6 kHz = a<br>9 kHz = b<br>11 kHz = ab  |
| <b>Dependant Variable 1:</b> Latency of occipital N40 trough during W<br><b>WS factor 1:</b> Stimulation frequency   | Effect of <b>Stimulation frequency</b><br>$H(3) = 3.22; p = 0.36$  | N/A                                                 |
| <b>Dependant Variable 1:</b> Latency of frontal N40 trough during SWS<br><b>WS factor 1:</b> Stimulation frequency   | Effect of <b>Stimulation frequency</b><br>$H(3) = 5.40; p = 0.14$  | N/A                                                 |
| <b>Dependant Variable 1:</b> Latency of occipital N40 trough during SWS<br><b>WS factor 1:</b> Stimulation frequency | Effect of <b>Stimulation frequency</b><br>$H(3) = 61.66; p < ****$ | 2 kHz = a<br>6 kHz = b<br>9 kHz = a<br>11 kHz = a   |
| <b>Dependant Variable 1:</b> Latency of frontal P120 peak during W<br><b>WS factor 1:</b> Stimulation frequency      | Effect of <b>Stimulation frequency</b><br>$H(3) = 4.96; p = 0.17$  | N/A                                                 |
| <b>Dependant Variable 1:</b> Latency of frontal P120 peak during SWS<br><b>WS factor 1:</b> Stimulation frequency    | Effect of <b>Stimulation frequency</b><br>$H(3) = 8.40; p < *$     | 2 kHz = ab<br>6 kHz = a<br>9 kHz = ab<br>11 kHz = b |

**Table C.8:** Statistical analyses for figure C.4

| Variables                                                                                             | Welch's t test                                  |
|-------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| <b>Dependant Variable 1:</b> Amplitude of frontal P20 peak<br><b>WS factor 1:</b> Vigilance state     | <b>W vs SWS</b><br>$t_{2717} = 6.64; p < ****$  |
| <b>Dependant Variable 1:</b> Amplitude of occipital P20 peak<br><b>WS factor 1:</b> Vigilance state   | <b>W vs SWS</b><br>$t_{4192} = 12.56; p < ****$ |
| <b>Dependant Variable 1:</b> Amplitude of frontal N40 trough<br><b>WS factor 1:</b> Vigilance state   | <b>W vs SWS</b><br>$t_{2144} = 19.25; p < ****$ |
| <b>Dependant Variable 1:</b> Amplitude of occipital N40 trough<br><b>WS factor 1:</b> Vigilance state | <b>W vs SWS</b><br>$t_{3165} = 3887; p < ****$  |
| <b>Dependant Variable 1:</b> Amplitude of frontal P120 peak<br><b>WS factor 1:</b> Vigilance state    | <b>W vs SWS</b><br>$t_{275} = 4.46; p < ****$   |

**Table C.9:** Statistical analyses for figure C.5

| Variables                                                                                           | Welch's t test                                  |
|-----------------------------------------------------------------------------------------------------|-------------------------------------------------|
| <b>Dependant Variable 1:</b> Latency of frontal P20 peak<br><b>WS factor 1:</b> Vigilance state     | <b>W vs SWS</b><br>$t_{1883} = 3.06; p < **$    |
| <b>Dependant Variable 1:</b> Latency of occipital P20 peak<br><b>WS factor 1:</b> Vigilance state   | <b>W vs SWS</b><br>$t_{3078} = 4.85; p < ****$  |
| <b>Dependant Variable 1:</b> Latency of frontal N40 trough<br><b>WS factor 1:</b> Vigilance state   | <b>W vs SWS</b><br>$t_{1647} = 13.59; p < ****$ |
| <b>Dependant Variable 1:</b> Latency of occipital N40 trough<br><b>WS factor 1:</b> Vigilance state | <b>W vs SWS</b><br>$t_{2403} = 3.34; p < ***$   |
| <b>Dependant Variable 1:</b> Latency of frontal P120 peak<br><b>WS factor 1:</b> Vigilance state    | <b>W vs SWS</b><br>$t_{1394} = 11.84; p < ****$ |

**Table C.10:** Statistical analyses for figure C.6

| Variables                                                                                                           | Comparison                                                                                         |
|---------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Amplitude of frontal P20 peak during PW<br><b>WS factor 1:</b> Stimulation frequency   | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 6.13$ ; $p = 0.11$      |
| <b>Dependant Variable 1:</b> Amplitude of occipital P20 peak during PW<br><b>WS factor 1:</b> Stimulation frequency | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$F_{3,450} = 1.46$ ; $p = 0.23$ |
| <b>Dependant Variable 1:</b> Amplitude of frontal N40 trough during PW<br><b>WS factor 1:</b> Stimulation frequency | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 0.81$ ; $p = 0.85$      |
| <b>Dependant Variable 1:</b> Amplitude of frontal P120 peak during PW<br><b>WS factor 1:</b> Stimulation frequency  | <i>One-way ANOVA</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 4.051$ ; $p = 0.26$      |

| Variables                                                                                           | t test                                                                                  |
|-----------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Amplitude of frontal P20 peak<br><b>WS factor 1:</b> Condition         | <i>Welch's t test</i><br><b>Vehicle vs 5-MeO-DMT</b><br>$t_{347.3} = 0.84$ ; $p = 0.40$ |
| <b>Dependant Variable 1:</b> Amplitude of occipital P20 peak<br><b>WS factor 1:</b> Vigilance state | <i>t test</i><br><b>Vehicle vs 5-MeO-DMT</b><br>$t_{601} = 0.45$ ; $p = 0.65$           |
| <b>Dependant Variable 1:</b> Amplitude of frontal N40 trough<br><b>WS factor 1:</b> Vigilance state | <i>Welch's t test</i><br><b>Vehicle vs 5-MeO-DMT</b><br>$t_{273.8} = 3.95$ ; $p < ****$ |
| <b>Dependant Variable 1:</b> Amplitude of frontal P120 peak<br><b>WS factor 1:</b> Vigilance state  | <i>t test</i><br><b>Vehicle vs 5-MeO-DMT</b><br>$t_{224} = 3.63$ ; $p < ***$            |

Table C.11: Statistical analyses for figure C.7

| Variables                                                                                                         | Kruskal-Wallis                                                                                            |
|-------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Latency of frontal P20 peak during PW<br><b>WS factor 1:</b> Stimulation frequency   | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 4.025$ ; $p = 0.26$            |
| <b>Dependant Variable 1:</b> Latency of occipital P20 peak during PW<br><b>WS factor 1:</b> Stimulation frequency | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 3.47$ ; $p = 0.32$             |
| <b>Dependant Variable 1:</b> Latency of frontal N40 trough during PW<br><b>WS factor 1:</b> Stimulation frequency | <i>Kruskal-Wallis</i><br>Effect of <b>Stimulation frequency</b><br>$H(3) = 1.88$ ; $p = 0.60$             |
| <b>Dependant Variable 1:</b> Latency of frontal P120 peak during PW<br><b>WS factor 1:</b> Stimulation frequency  | <i>Brown-Forsythe ANOVA</i><br>Effect of <b>Stimulation frequency</b><br>$F_{3,100} = 0.099$ ; $p = 0.96$ |

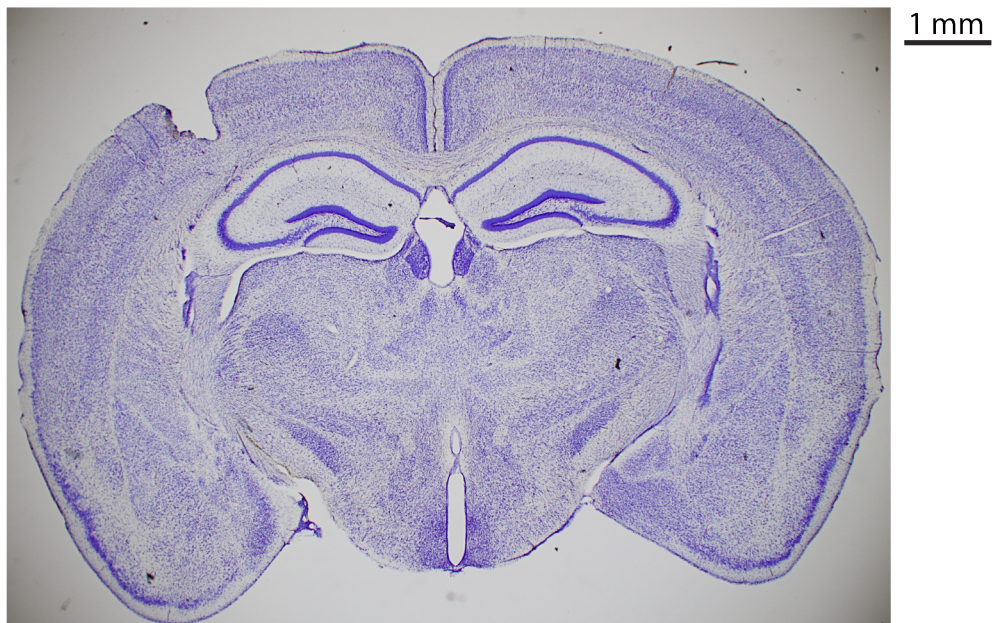
| Variables                                                                                         | t test                                                                                   |
|---------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Latency of frontal P20 peak<br><b>WS factor 1:</b> Condition         | <i>Welch's t test</i><br><b>Vehicle vs 5-MeO-DMT</b><br>$t_{264.9} = 1.82$ ; $p = 0.069$ |
| <b>Dependant Variable 1:</b> Latency of occipital P20 peak<br><b>WS factor 1:</b> Vigilance state | <i>t test</i><br><b>Vehicle vs 5-MeO-DMT</b><br>$t_{601} = 0.51$ ; $p = 0.61$            |
| <b>Dependant Variable 1:</b> Latency of frontal N40 trough<br><b>WS factor 1:</b> Vigilance state | <i>t test</i><br><b>Vehicle vs 5-MeO-DMT</b><br>$t_{311} = 0.31$ ; $p = 0.75$            |
| <b>Dependant Variable 1:</b> Latency of frontal P120 peak<br><b>WS factor 1:</b> Vigilance state  | <i>t test</i><br><b>Vehicle vs 5-MeO-DMT</b><br>$t_{224} = 2.22$ ; $p < *$               |



# D

## Supplementary materials of Chapter 6

### D.1 Histology



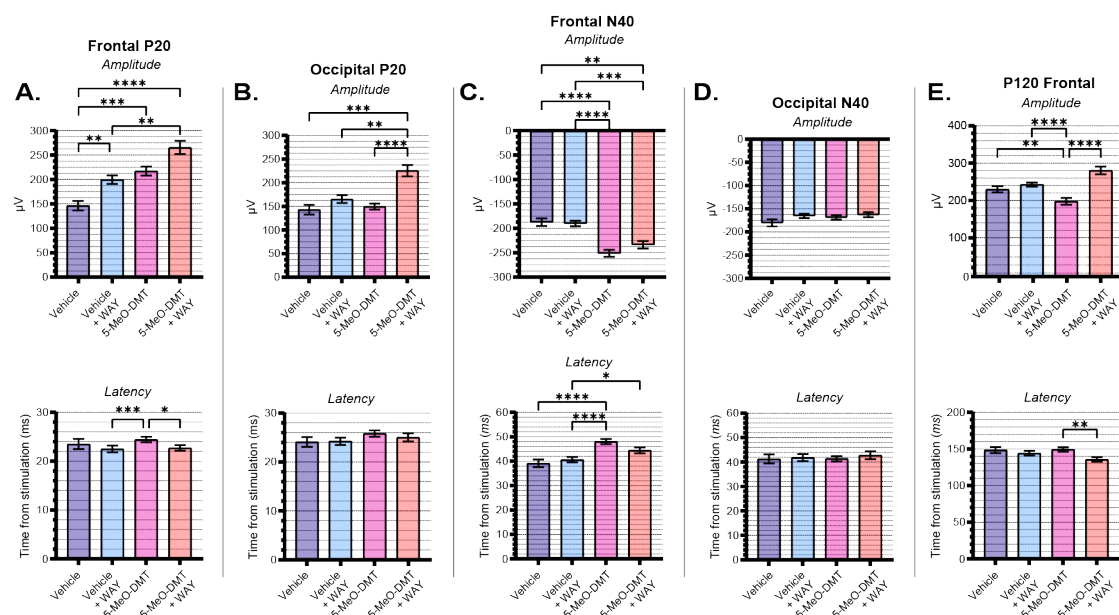
**Figure D.1: Histology results of Chapter 6.** Representative histology picture in an animal implanted intracortically with a cannula. Image acquired on an olympus on a brain stained with cresyl violet. Brain slice was located at -1.9 antero-posterior. The cannula track is located at the junction of the barrel field in the primary somatosensory cortex and the posterior parietal cortex.

## **D.2 5-HT<sub>1A</sub> antagonist effects on auditory ERP components**

The injection of WAY alongside vehicle showed that WAY induced a significant increase of the P20 amplitude, reaching 5-MeO-DMT-like levels (Kruskal-Wallis  $H_3 = 39.19$ ,  $p < 0.0001$ ; Dunn's multiple comparison  $p < 0.01$ ) (Figure D.2.A). Similarly, 5-MeO-DMT+WAY condition tended to show a bigger P20 than 5-MeO-DMT alone, but no significant difference was found (Dunn's multiple comparison  $p = 0.06$ ) (Figure 9D.2.A). The latency of the frontal P20 was unaffected by WAY alone, as suggested by the latency of vehicle response being similar to the one of vehicle+WAY (Kruskal-Wallis  $H_3 = 19.38$ ,  $p < 0.0001$  ; Dunn's multiple comparison  $p = 0.99$ ) (Figure D.2.A). 5-MeO-DMT alone induced a minor delay in P20 compared to vehicle+WAY, but not vehicle (Dunn's multiple comparison  $p < 0.001$ ). The injection of WAY with 5-MeO-DMT however restored the latency to vehicle-like levels, and significantly different to 5-MeO-DMT alone (Dunn's multiple comparison  $p < 0.05$ ) (Figure D.2.A).

In the occipital derivation, no difference was found in the amplitude of P20 between the vehicle, the vehicle+WAY and the 5-MeO-DMT condition (Kruskal-Wallis  $H_3 = 19.38$ ,  $p < 0.0001$  ; Dunn's multiple comparison  $p > 0.05$ ) (Figure D.2.A). 5-MeO-DMT+WAY, however, elicited an occipital P20 of much higher amplitude than all three other conditions (Dunn's multiple comparison  $p < 0.01$ ) (Figure D.2.B). Regarding the latency, P20 occurred at around the same time after the stimulation for all conditions (Kruskal Wallis  $H_3 = 4.05$ ,  $p = 0.26$ ) (Figure D.2.B).

Regarding N40, the addition of WAY did not change the amplitude of the response in the vehicle or 5-MeO-DMT conditions, with the 5-MeO-DMT and 5-MeO-DMT+WAY conditions having a significantly deeper trough than the vehicle conditions (Kruskal-Wallis  $H_3 = 50.86$ ,  $p < 0.0001$ , Dunn's multiple comparison  $p < 0.01$  against both vehicle conditions). (Figure D.2.C). Similar observations can be made about the latency of frontal N40 where vehicle and vehicle+WAY



**Figure D.2: EEG ERP component changes with the presence of a 5-HT<sub>1A</sub> receptor antagonist.** A-B. Analysis of P20 amplitude (top) and latency (bottom) in the frontal (A.) and occipital (B.) EEG derivation. C-D. Analysis of N40 amplitude (top) and latency (bottom) in the frontal (C) and occipital (D) EEG derivation. E. Analysis of P120 amplitude (top) and latency (bottom) in the frontal EEG derivation. There is no occipital visualisation because of the lack of clear P120 component in the occipital derivation during wake and sleep. Data are presented as mean + SEM (\*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p < 0.001). N = 3 for all panels. See Table D.11 for statistical analyses.

conditions are not different from one another (Kruskal-Wallis  $H_3 = 33.15$ ,  $p < 0.0001$ ; Dunn's multiple comparison  $p = 0.99$ ) The injection of 5-MeO-DMT significantly increased the latency (Dunn's multiple comparison  $p < 0.0001$  against both vehicle conditions). 5-MeO-DMT+WAY had a significantly higher latency to N40 compared to vehicle+WAY (Dunn's multiple comparison  $p < 0.05$ ) but not compared to vehicle alone and 5-MeO-DMT alone. No significant difference was found between any of the conditions regarding N40 amplitude and latency to N40 in the occipital derivation (Figure D.2.D).

Regarding the frontal P120, 5-MeO-DMT+WAY had an amplitude similar to that of vehicle and vehicle+WAY conditions, and significantly higher than in the 5-MeO-DMT alone condition (Kruskal-Wallis  $H_3 = 49.18$ ,  $p < 0.0001$ , Dunn's multiple comparison  $p < 0.01$ ) (Figure D.2.E). In terms of latency, the frontal

P120 occurred at the same time for the vehicle conditions and 5-MeO-DMT alone, but the addition of WAY to 5-MeO-DMT induced an earlier P120 than in 5-MeO-DMT alone stimulations (Kruskal-Wallis  $H_3 = 12.02$ ,  $p < 0.01$ ; Dunn's multiple comparison  $p < 0.01$ ) (Figure D.2.E).

## **D.3 Analysis tables**

Analysis tables of Chapter 6 are detailed on the next page.

**Table D.1:** Statistical analyses for figure 6.3.A-B & 6.4.A-B

| Variables                                                                                                                                                                                                | ANOVA with repeated measures                                                                                                                                                                                                                               | Multiple comparison (Fisher's LSD)                                                                                                                                                                                                                                                                               |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Log transformed frontal EEG power density for each frequency bins (Freq = 0.5:0.25:20) during W<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition   | <i>ME analysis</i><br>Effect of <b>Frequency</b><br>$F_{78,546} = 266.7; p < ****$<br><br>Effect of <b>Condition</b><br>$F_{2,4} = 2.062; p = 0.17$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{66,403} = 7.40; p < ****$                         | <b>Vehicle vs 5-MeO-DMT</b><br>[0.75:5.25]; 18.5<br><br><b>Vehicle vs 5-MeO-DMT + SD</b><br>[0.75:5.25]; 18.5<br><br><b>5-MeO-DMT vs 5-MeO-DMT + SD</b><br>$p > 0.05$                                                                                                                                            |
| <b>Dependant Variable 1:</b> Log transformed occipital EEG power density for each frequency bins (Freq = 0.5:0.25:20) during W<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition | <i>Two-way ANOVA with repeated measures</i><br>Effect of <b>Frequency</b><br>$F_{78,468} = 76.5; p < ****$<br><br>Effect of <b>Condition</b><br>$F_{2,2} = 0.22; p = 0.80$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{26,936} = 10.39; p < ****$ | <b>Vehicle vs 5-MeO-DMT</b><br>[0.75:5.5]; [6.25:11.5]; 12.75; 19.25<br><br><b>Vehicle vs 5-MeO-DMT + SD</b><br>[0.75:11.5]; [2.75:5.5]; [6.5:11.5]; [12.25:12.75]<br><br><b>5-MeO-DMT vs 5-MeO-DMT + SD</b><br>[0.75:2.5]                                                                                       |
| <b>Dependant Variable 1:</b> Log transformed frontal EEG power density for each frequency bins (Freq = 0.5:0.25:20) during SWS<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition | <i>ME analysis</i><br>Effect of <b>Frequency</b><br>$F_{78,546} = 409.8; p < ****$<br><br>Effect of <b>Condition</b><br>$F_{3,21} = 5.21; p < **$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{234,1476} = 6.82; p < ****$                         | <b>Vehicle vs Vehicle + SD</b><br>[0.75:3.5]<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[7:16.25]; 16.75; 18.75; 19.25; 19.75<br><br><b>Vehicle vs 5-MeO-DMT + SD</b><br>[0.75:4]; 7.25; 19.25; 19.75<br><br><b>Vehicle + SD vs 5-MeO-DMT</b><br>[0.75:3]; 5.5; [13.25:16.25]; [16.75:17]; [17.5:17.75]; [18.25:18.5] |

|                                                                                                                                                                                                            |                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                           |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                             | <b>Vehicle + SD vs 5-MeO-DMT + SD</b><br>12.25<br><br><b>5-MeO-DMT vs 5-MeO-DMT + SD</b><br>[0.75:3.5]; [5.5:7.75];<br>[8.75:11.25]; [12:12.25];<br>[12.75:13]; 16.25; 18.25                                                                                                                                                                                                                              |
| <b>Dependant Variable 1:</b> Log transformed occipital EEG power density for each frequency bins (Freq = 0.5:0.25:20) during SWS<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition | <i>ME analysis</i><br>Effect of <b>Frequency</b><br>$F_{17,462} = 330.5$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{3,8} = 0.89$ ; $p = 0.47$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{231,308} = 3.15$ ; $p < ****$ | <b>Vehicle vs Vehicle + SD</b><br>[0.75:2]<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[8.75:10.75]; [12:12.5]<br><br><b>Vehicle vs 5-MeO-DMT + SD</b><br>2; [2.75:3.5]<br><br><b>Vehicle + SD vs 5-MeO-DMT</b><br>[1:2.75]; [8.75:10:75];<br>[12:12.5]<br><br><b>Vehicle + SD vs 5-MeO-DMT + SD</b><br>$p > 0.05$<br><br><b>5-MeO-DMT vs 5-MeO-DMT + SD</b><br>1.25; 3.25; 6.5; 8.25;<br>[8.75:11]; [12:12.25] |

**Table D.2:** Statistical analyses for figure 6.3.D-F

| Variables                                                                                                                                                                                    | Mixed-effects analysis                                                                                                                                                                            | Multiple comparison<br>(Fisher's LSD)                                                                                                                             |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Average area under curve of log transformed frontal EEG power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition   | <b>Effect of Time</b><br>$F_{28, 968} = 1.92; p < **$<br><br><b>Effect of Condition</b><br>$F_{2, 9} = 3.34; p = 0.07$<br><br><b>Effect of Time x Condition</b><br>$F_{56, 217} = 1.00; p = 0.48$ | N/A                                                                                                                                                               |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed Occipital EEG power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition | <b>Effect of Time</b><br>$F_{30, 80} = 1.91; p < **$<br><br><b>Effect of Condition</b><br>$F_{2, 9} = 0.23; p = 0.80$<br><br><b>Effect of Time x Condition</b><br>$F_{60, 210} = 1.41; p < *$     | <b>Vehicle vs 5-MeO-DMT</b><br>$p > 0.05$<br><br><b>Vehicle vs 5-MeO-DMT + SD</b><br>60<br><br><b>5-MeO-DMT vs 5-MeO-DMT + SD</b><br>[48:50]                      |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed Occipital EEG power density between 6 - 10 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition  | <b>Effect of Time</b><br>$F_{20, 20} = 7.54; p < ****$<br><br><b>Effect of Condition</b><br>$F_{2, 9} = 2.47; p = 0.13$<br><br><b>Effect of Time x Condition</b><br>$F_{40, 92} = 3.29; p < ****$ | <b>Vehicle vs 5-MeO-DMT</b><br>[2:34]<br><br><b>Vehicle vs 5-MeO-DMT + SD</b><br>[2:12]; [16:22]<br><br><b>5-MeO-DMT vs 5-MeO-DMT + SD</b><br>18; [32:36]; 40; 46 |

**Table D.3:** Statistical analyses for figure 6.4.C-E

| Variables                                                                                                                                                             | Two-way ANOVA with repeated measures                                                                                                                                                                                  | Multiple comparison (Šidák's)                                          |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Time to first consolidate episode of SWS<br><br><b>WS factor 1:</b> Injection<br><br><b>WS factor 2:</b> Sleep deprivation               | Effect of <b>Injection</b><br>$F_{1,7} = 85.41; p < ****$<br><br>Effect of <b>Sleep deprivation</b><br>$F_{1,7} = 9.17; p < *$<br><br>Effect of <b>Injection x Sleep deprivation</b><br>$F_{1,7} = 7.11; p < *$       | Vehicle = a<br>Vehicle + SD = a<br>5-MeO-DMT = b<br>5-MeO-DMT + SD = c |
| <b>Dependant Variable 1:</b> Time to first consolidate episode of PS<br><br><b>WS factor 1:</b> Injection<br><br><b>WS factor 2:</b> Sleep deprivation                | Effect of <b>Injection</b><br>$F_{1,7} = 350.2; p < ****$<br><br>Effect of <b>Sleep deprivation</b><br>$F_{1,7} = 10.40; p < *$<br><br>Effect of <b>Injection x Sleep deprivation</b><br>$F_{1,7} = 11.06; p < *$     | Vehicle = a<br>Vehicle + SD = a<br>5-MeO-DMT = b<br>5-MeO-DMT + SD = c |
| <b>Dependant Variable 1:</b> Time from SWS onset to first consolidate episode of PS<br><br><b>WS factor 1:</b> Injection<br><br><b>WS factor 2:</b> Sleep deprivation | Effect of <b>Injection</b><br>$F_{1,7} = 25.21; p < ****$<br><br>Effect of <b>Sleep deprivation</b><br>$F_{1,7} = 1.72; p = 0.23$<br><br>Effect of <b>Injection x Sleep deprivation</b><br>$F_{1,7} = 0.13; p = 0.73$ | N/A                                                                    |

**Table D.4:** Statistical analyses for figure 6.5.A

| Variables                                                                                   | paired t test                                         |
|---------------------------------------------------------------------------------------------|-------------------------------------------------------|
| <b>Dependant Variable 1:</b> Time spent in wakefulness<br><br><b>WS factor 1:</b> Condition | <b>Vehicle vs 5-MeO-DMT</b><br>$t_3 = 0.42; p = 0.70$ |



**Table D.5:** Statistical analyses for figure 6.5.B-F

| Variables                                                                                                                                                                                                      | ANOVA with repeated measures                                                                                                                                                                                                                                                            | Multiple comparison (Fisher's LSD)                                                                                                                                                                                                                               | Multiple comparison (Tukey's)                                                                                                                        |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Log transformed frontal EEG power density for each frequency bins (Freq = 0.5:0.25:20) during W<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition         | <i>ME analysis</i><br>Effect of <b>Frequency</b><br>$F_{78,234} = 24.03$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{2,6} = 0.28$ ; $p = 0.77$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{156,231} = 0.93$ ; $p = 0.68$                                             | N/A                                                                                                                                                                                                                                                              | N/A                                                                                                                                                  |
| <b>Dependant Variable 1:</b> Log transformed occipital right EEG power density for each frequency bins (Freq = 0.5:0.25:20) during W<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition | <i>Two-way ANOVA with repeated measures</i><br>Effect of <b>Frequency</b><br>$F_{78,234} = 37.59$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{2,6} = 0.086$ ; $p = .092$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{156,468} = 1.33$ ; $p < *$                      | <b>Vehicle vs 5-MeO-DMT</b><br>[0.75:1.25]; 2; 8.25;<br>[9.5:9.75]<br><br><b>Vehicle vs 5-MeO-DMT + SD</b><br>[0.75:1.25]; [6.25:7.25]<br><br><b>5-MeO-DMT vs 5-MeO-DMT + SD</b><br>[8.75:10.5]                                                                  | N/A                                                                                                                                                  |
| <b>Dependant Variable 1:</b> Log transformed occipital left EEG power density for each frequency bins (Freq = 0.5:0.25:20) during W<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition  | <i>Two-way ANOVA with repeated measures</i><br>Effect of <b>Frequency</b><br>$F_{78,231} = 67.65$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{2,6} = 1.44$ ; $p = 0.31$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{154,462} = 1.90$ ; $p < ****$                    | <b>Vehicle vs 5-MeO-DMT</b><br>3.5; 4; [4.5:5.25]; 6.5;<br>[15:15.25]; 17.5<br><br><b>Vehicle vs 5-MeO-DMT + SD</b><br>[8.25:12]; [13.5:13.75];<br>[15:15.25]; [17.25:17.5]<br><br><b>5-MeO-DMT vs 5-MeO-DMT + SD</b><br>[4.5:5.25]; 6.25; [8.5:10.75];<br>16.25 | N/A                                                                                                                                                  |
| <b>Dependant Variable 1:</b> Log transformed superior LFP power density for each frequency bins (Freq = 0.5:0.25:20) during W<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition        | <i>Two-way ANOVA with repeated measures</i><br>Effect of <b>Frequency</b><br>$F_{78,234} = 24.36$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{2,6} = 6.09$ ; $p < *$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{156,468} = 0.68$ ; $p = 0.99$                       | N/A                                                                                                                                                                                                                                                              | <b>Vehicle vs 5-MeO-DMT</b><br>$p = 0.58$<br><br><b>Vehicle vs 5-MeO-DMT + SD</b><br>$p < *$<br><br><b>5-MeO-DMT vs 5-MeO-DMT + SD</b><br>$p = 0.12$ |
| <b>Dependant Variable 1:</b> Log transformed inferior LFP power density for each frequency bins (Freq = 0.5:0.25:20) during W<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition        | <i>Two-way ANOVA with repeated measures</i><br>Effect of <b>Frequency</b><br>$F_{78,234} = 51.37$ ; $p < ****$ (GG)<br><br>Effect of <b>Condition</b><br>$F_{112,336} = 3.99$ ; $p = 0.08$ (GG)<br><br>Effect of <b>Frequency x Condition</b><br>$F_{168,504} = 0.67$ ; $p = 0.99$ (GG) | N/A                                                                                                                                                                                                                                                              | N/A                                                                                                                                                  |

**Table D.6:** Statistical analyses for figure 6.5.G-J

| Variables                                                                                                                                                                                   | Mixed-effects analysis                                                                                                                                                                                   |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Average area under curve of log transformed frontal EEG power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition  | Effect of <b>Time</b><br>$F_{29,87} = 0.74$ ; $p = 0.82$<br><br>Effect of <b>Condition</b><br>$F_{2,6} = 0.04$ ; $p = 0.96$<br><br>Effect of <b>Time x Condition</b><br>$F_{58,92} = 0.96$ ; $p = 0.58$  |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed frontal EEG power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition  | Effect of <b>Time</b><br>$F_{28,84} = 1.01$ ; $p = 0.38$<br><br>Effect of <b>Condition</b><br>$F_{2,6} = 0.12$ ; $p = 0.89$<br><br>Effect of <b>Time x Condition</b><br>$F_{56,56} = 1.20$ ; $p = 0.24$  |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed superior LFP power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition | Effect of <b>Time</b><br>$F_{26,78} = 1.50$ ; $p = 0.09$<br><br>Effect of <b>Condition</b><br>$F_{2,6} = 0.54$ ; $p = 0.61$<br><br>Effect of <b>Time x Condition</b><br>$F_{52,51} = 1.43$ ; $p = 0.10$  |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed inferior LFP power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition | Effect of <b>Time</b><br>$F_{27,81} = 1.45$ ; $p = 0.10$<br><br>Effect of <b>Condition</b><br>$F_{2,6} = 0.005$ ; $p = 0.99$<br><br>Effect of <b>Time x Condition</b><br>$F_{54,52} = 1.47$ ; $p = 0.08$ |

**Table D.7:** Statistical analyses for figure 6.6

| Variables                                                                                                                                                                                                        | ANOVA with repeated measures                                                                                                                                                                                                                  | Multiple comparison (Fisher's LSD)                                                                                                                                                                                                                                                                                                                                                                                  |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Log transformed frontal EEG power density for each frequency bins (Freq = 0.5:0.25:20) during W<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition           | <i>ME analysis</i><br>Effect of <b>Frequency</b><br>$F_{78,468} = 834.8$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{3,8} = 2.39$ ; $p = 0.10$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{234,1009} = 11.49$ ; $p < ****$ | <b>Vehicle vs Vehicle + WAY100635</b><br>$p > 0.05$<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[0.75:5]<br><br><b>Vehicle vs 5-MeO-DMT + WAY100635</b><br>[0.75:6]<br><br><b>Vehicle + WAY100635 vs 5-MeO-DMT</b><br>[0.75:5]; [7.5:8.5]; [13.25:13.75]<br><br><b>Vehicle + WAY100635 vs 5-MeO-DMT + WAY100635</b><br>[0.75:6.25]; 13.5<br><br><b>5-MeO-DMT vs 5-MeO-DMT + WAY100635</b><br>1.5; [3:4.75]; 6.5; [7:7.75] |
| <b>Dependant Variable 1:</b> Log transformed occipital right EEG power density for each frequency bins (Freq = 0.5:0.25:20) during SWS<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition | <i>ME analysis</i><br>Effect of <b>Frequency</b><br>$F_{78,468} = 42.68$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{3,8} = 2.01$ ; $p = 0.15$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{234,851} = 13.31$ ; $p < ****$  | <b>Vehicle vs Vehicle + WAY100635</b><br>[6.5:7]<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[0.75:4.75]; [6.25:9];<br><br><b>Vehicle vs 5-MeO-DMT + WAY100635</b><br>[0.75:5]; [6.75:8.5]<br><br><b>Vehicle + WAY100635 vs 5-MeO-DMT</b><br>[0.75:4.75]; [6.75:9]<br><br><b>Vehicle + WAY100635 vs 5-MeO-DMT + WAY100635</b><br>[0.75:5.5]; [7.75:8.25]                                                                  |

|                                                                                                                                                                                                |                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                                                                                                                                |                                                                                                                                                                                                                                                                     | <b>5-MeO-DMT vs 5-MeO-DMT + WAY100635</b><br>[0.75:2.25]; 3; [3.5:4.5];<br>5.75; [6.5:7.75]                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Dependant Variable 1:</b> Log transformed LFP power density for each frequency bins (Freq = 0.5:0.25:20) during W<br><br><b>WS factor 1:</b> Frequency<br><br><b>WS factor 2:</b> Condition | <i>Two-way ANOVA with repeated measures</i><br>Effect of <b>Frequency</b><br>$F_{78,156} = 616.2$ ; $p < ****$<br><br>Effect of <b>Condition</b><br>$F_{3,6} = 10.81$ ; $p < **$<br><br>Effect of <b>Frequency x Condition</b><br>$F_{234,468} = 5.58$ ; $p < ****$ | <b>Vehicle vs Vehicle + WAY100635</b><br>[1.5:1.75]; [2.25:4]; 13<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[0.75:5.5]; [6.5:9.25];<br><br><b>Vehicle vs 5-MeO-DMT + WAY100635</b><br>[0.75:5.25]; [11.75:17.5]; [18:18.5]<br><br><b>Vehicle + WAY100635 vs 5-MeO-DMT</b><br>[0.75:5.5]; [7:9.25]<br><br><b>Vehicle + WAY100635 vs 5-MeO-DMT + WAY100635</b><br>[0.75:6]; [12:12.75]; [13.5:14.5]; 18.5<br><br><b>5-MeO-DMT vs 5-MeO-DMT + WAY100635</b><br>[6.25:8.75]; [9.25:9.5]; [11.75:12.25]; [12.75:14]; 15.25; 16; [16.5:17.25]; [18:18.5] |

**Table D.8:** Statistical analyses for figure 6.7.A-D

| Variables                                                                                                                                                                                    | Mixed-effects analysis                                                                                                                                                                                                                                                                        | Multiple comparison<br>(Fisher's LSD)                                                                                                                                                                                                                                                                                                                                                                                 |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Average area under curve of log transformed frontal EEG power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition   | <p>Effect of <b>Time</b><br/> <math>F_{28, 940} = 4.01</math>; <math>p &lt; ****</math></p> <p>Effect of <b>Condition</b><br/> <math>F_{3, 6} = 3.16</math>; <math>p = 0.06</math></p> <p>Effect of <b>Time x Condition</b><br/> <math>F_{84, 96} = 2.37</math>; <math>p &lt; ****</math></p> | <p><b>Vehicle vs Vehicle + WAY100635</b><br/> <math>p &gt; 0.05</math></p> <p><b>Vehicle vs 5-MeO-DMT</b><br/> [2:12]</p> <p><b>Vehicle vs 5-MeO-DMT + WAY100635</b><br/> [6:10]; [16:28]</p> <p><b>Vehicle + WAY100635 vs 5-MeO-DMT</b><br/> [4:12]</p> <p><b>Vehicle + WAY100635 vs 5-MeO-DMT + WAY100635</b><br/> [8:10]; [16:30]</p> <p><b>5-MeO-DMT vs 5-MeO-DMT + WAY100635</b><br/> [4:6]; 16; [20:30]; 54</p> |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed occipital EEG power density between 0.5 - 4 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition | <p>Effect of <b>Time</b><br/> <math>F_{28, 82} = 4.18</math>; <math>p &lt; ****</math></p> <p>Effect of <b>Condition</b><br/> <math>F_{3, 21} = 3.77</math>; <math>p &lt; *</math></p> <p>Effect of <b>Time x Condition</b><br/> <math>F_{78, 16} = 2.01</math>; <math>p &lt; ***</math></p>  | <p><b>Vehicle vs Vehicle + WAY100635</b><br/> <math>p &gt; 0.05</math></p> <p><b>Vehicle vs 5-MeO-DMT</b><br/> [6:12]; 16; [22:24]; 34</p> <p><b>Vehicle vs 5-MeO-DMT + WAY100635</b><br/> [6:16]; [20:22]; [28:30]</p> <p><b>Vehicle + WAY100635 vs 5-MeO-DMT</b><br/> [6:12]; 16; [30:32]</p> <p><b>Vehicle + WAY100635 vs 5-MeO-DMT + WAY100635</b><br/> [6:16]; [20:22]; [28:30]</p>                              |

|                                                                                                                                                                                             |                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                          |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                                                                                                                             |                                                                                                                                                                                                            | <b>5-MeO-DMT vs 5-MeO-DMT + WAY100635</b><br>4; 16; 22; [28:30]; 34; 38                                                                                                                                                                                                                                                                                                  |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed occipital EEG power density between 6 - 10 Hz<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition | <b>Effect of Time</b><br>$F_{29,203} = 0.98$ ; $p = 0.50$<br><br><b>Effect of Condition</b><br>$F_{3,21} = 4.03$ ; $p < *$<br><br><b>Effect of Time x Condition</b><br>$F_{87,443} = 2.92$ ; $p < ****$    | <b>Vehicle vs Vehicle + WAY100635</b><br>$p > 0.05$<br><br><b>Vehicle vs 5-MeO-DMT</b><br>2; [4:14]; [18:24]; 36<br><br><b>Vehicle vs 5-MeO-DMT + WAY100635</b><br>16; 28<br><br><b>Vehicle + WAY100635 vs 5-MeO-DMT</b><br>[02]; [6:24]<br><br><b>Vehicle + WAY100635 vs 5-MeO-DMT + WAY100635</b><br>16; 28<br><br><b>5-MeO-DMT vs 5-MeO-DMT + WAY100635</b><br>[2:36] |
| <b>Dependant Variable 1:</b> Pupil diameter<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition                                                                            | <b>Effect of Time</b><br>$F_{40,200} = 8.76$ ; $p < ****$<br><br><b>Effect of Condition</b><br>$F_{3,15} = 0.68$ ; $p = 0.58$<br><br><b>Effect of Time x Condition</b><br>$F_{120,401} = 1.64$ ; $p < ***$ | <b>Vehicle vs Vehicle + WAY100635</b><br>$p > 0.05$<br><br><b>Vehicle vs 5-MeO-DMT</b><br>[4:12]<br><br><b>Vehicle vs 5-MeO-DMT + WAY100635</b><br>$p > 0.05$<br><br><b>Vehicle + WAY100635 vs 5-MeO-DMT</b><br>16<br><br><b>Vehicle + WAY100635 vs 5-MeO-DMT + WAY100635</b><br>$p > 0.05$<br><br><b>5-MeO-DMT vs 5-MeO-DMT + WAY100635</b><br>[8:10]                   |

**Table D.9:** Statistical analyses for figure 6.7.E-F

| Variables                                                                                                                                                                                                                                               | Simple Linear Regression                                                                                         |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Average area under curve of log transformed frontal EEG power density between 0.5 - 4 Hz following an injection of 5-MeO-DMT<br><br><b>Dependant Variable 2:</b> Pupil diameter<br><br><b>WS factor 1:</b> Time            | Slope = $0.35 \pm 0.05$<br>$r^2 = 0.60$<br><br><i>Line different from zero</i><br>$F_{129} = 42.78$ ; $p < ****$ |
| <b>Dependant Variable 1:</b> Average area under curve of log transformed frontal EEG power density between 6 - 10 Hz following an injection of 5-MeO-DMT + WAY100635<br><br><b>Dependant Variable 2:</b> Pupil diameter<br><br><b>WS factor 1:</b> Time | Slope = $0.03 \pm 0.04$<br>$r^2 = 0.01$<br><br><i>Line different from zero</i><br>$F_{127} = 0.38$ ; $p = 0.54$  |

**Table D.10:** Statistical analyses for figure 6.8

| Variables                                                                                                                                                                                       | 2-way ANOVA with repeated measures                                                                                                                                                                                                |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Frontal EEG activity during W around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition   | Effect of <b>Time</b><br>$F_{\text{53, 53}} = 3.27$ ; $p = 0.32$ (GG)<br><br>Effect of <b>Condition</b><br>$F_{3,3} = 10.14$ ; $p = 0.19$ (GG)<br><br>Effect of <b>Time x Condition</b><br>$F_{459,459} = 1.81$ ; $p = 0.41$ (GG) |
| <b>Dependant Variable 1:</b> Occipital EEG activity during W around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition | Effect of <b>Time</b><br>$F_{\text{53, 53}} = 2.26$ ; $p = 0.37$ (GG)<br><br>Effect of <b>Condition</b><br>$F_{3,3} = 2.65$ ; $p = 0.35$ (GG)<br><br>Effect of <b>Time x Condition</b><br>$F_{459,459} = 2.20$ ; $p = 0.38$ (GG)  |
| <b>Dependant Variable 1:</b> LFP activity during W around an auditory stimulation (Time = -198.219:3.91:400.011)<br><br><b>WS factor 1:</b> Time<br><br><b>WS factor 2:</b> Condition           | Effect of <b>Time</b><br>$F_{\text{53,306}} = 4.97$ ; $p = 0.088$ (GG)<br><br>Effect of <b>Condition</b><br>$F_{3,6} = 0.78$ ; $p = 0.49$ (GG)<br><br>Effect of <b>Time x Condition</b><br>$F_{459,918} = 1.63$ ; $p = 0.31$ (GG) |

Table D.11: Statistical analyses for figure D.2

| Variables                                                                                                            | Kruskal-Wallis                                                     | Multiple comparison (Dunn's)                                                           |
|----------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| <b>Dependant Variable 1:</b> Amplitude of frontal P20 peak during W<br><b>WS factor 1:</b> Stimulation frequency     | Effect of <b>Stimulation frequency</b><br>$H(3) = 39.19; p < ****$ | Vehicle = a<br>Vehicle + WAY100635 = b<br>5-MeO-DMT = bc<br>5-MeO-DMT + WAY100635 = c  |
| <b>Dependant Variable 1:</b> Amplitude of occipital P20 peak during W<br><b>WS factor 1:</b> Stimulation frequency   | Effect of <b>Stimulation frequency</b><br>$H(3) = 37.14; p < ****$ | Vehicle = a<br>Vehicle + WAY100635 = a<br>5-MeO-DMT = a<br>5-MeO-DMT + WAY100635 = b   |
| <b>Dependant Variable 1:</b> Latency of frontal P20 peak during W<br><b>WS factor 1:</b> Stimulation frequency       | Effect of <b>Stimulation frequency</b><br>$H(3) = 19.38; p < ***$  | Vehicle = ab<br>Vehicle + WAY100635 = a<br>5-MeO-DMT = b<br>5-MeO-DMT + WAY100635 = a  |
| <b>Dependant Variable 1:</b> Latency of occipital P20 peak during W<br><b>WS factor 1:</b> Stimulation frequency     | Effect of <b>Stimulation frequency</b><br>$H(3) = 4.051; p = 0.26$ | N/A                                                                                    |
| <b>Dependant Variable 1:</b> Amplitude of frontal N40 trough during W<br><b>WS factor 1:</b> Stimulation frequency   | Effect of <b>Stimulation frequency</b><br>$H(3) = 50.86; p < ****$ | Vehicle = a<br>Vehicle + WAY100635 = a<br>5-MeO-DMT = b<br>5-MeO-DMT + WAY100635 = b   |
| <b>Dependant Variable 1:</b> Amplitude of occipital N40 trough during W<br><b>WS factor 1:</b> Stimulation frequency | Effect of <b>Stimulation frequency</b><br>$H(3) = 3.79; p = 0.29$  | N/A                                                                                    |
| <b>Dependant Variable 1:</b> Latency of frontal N40 trough during W<br><b>WS factor 1:</b> Stimulation frequency     | Effect of <b>Stimulation frequency</b><br>$H(3) = 33.15; p < ****$ | Vehicle = ab<br>Vehicle + WAY100635 = a<br>5-MeO-DMT = c<br>5-MeO-DMT + WAY100635 = bc |
| <b>Dependant Variable 1:</b> Latency of occipital N40 trough during W<br><b>WS factor 1:</b> Stimulation frequency   | Effect of <b>Stimulation frequency</b><br>$H(3) = 0.95; p = 0.81$  | N/A                                                                                    |
| <b>Dependant Variable 1:</b> Amplitude of frontal P120 peak during W<br><b>WS factor 1:</b> Stimulation frequency    | Effect of <b>Stimulation frequency</b><br>$H(3) = 49.18; p < ****$ | Vehicle = a<br>Vehicle + WAY100635 = a<br>5-MeO-DMT = b<br>5-MeO-DMT + WAY100635 = a   |
| <b>Dependant Variable 1:</b> Latency of frontal P120 peak during W<br><b>WS factor 1:</b> Stimulation frequency      | Effect of <b>Stimulation frequency</b><br>$H(3) = 12.02; p < **$   | Vehicle = ab<br>Vehicle + WAY100635 = ab<br>5-MeO-DMT = a<br>5-MeO-DMT + WAY100635 = b |



## References

- Adamantidis, A. R., Gutierrez Herrera, C., & Gent, T. C. (2019). Oscillating circuitries in the sleeping brain. *Nat. Rev. Neurosci.*, *20*, 746–762.
- Adrian, E. D., & Matthews, B. H. (1934). The interpretation of potential waves in the cortex. *J. Physiol.*, *81*, 440–471.
- Adrian, E. D., & Yamagiwa, K. (1935). The origin of the berger rhythm. *Brain*, *58*, 323–351.
- Aghajanian, G. K. (1995). Electrophysiology of serotonin receptor subtypes and signal transduction pathways. In F. E. Bloom & D. J. Kupfer (Eds.), *Psychopharmacology – 4th generation of progress*. Raven Press.
- Aghajanian, G. K., & Marek, G. J. (1997). Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. *Neuropharmacology*, *36*, 589–599.
- Al-Imam, A. (2017). Could hallucinogens induce permanent pupillary changes in (ab)users? a case report from new zealand. *Case Rep. Neurol. Med.*, *2017*, 2503762.
- Andrillon, T., Burns, A., Mackay, T., Windt, J., & Tsuchiya, N. (2021). Predicting lapses of attention with sleep-like slow waves. *Nature Communications* *2021 12:1*, *12*, 1–12.
- Arzi, A., Shedlesky, L., Ben-Shaul, M., Nasser, K., Oksenberg, A., Hairston, I. S., & Sobel, N. (2012). Humans can learn new information during sleep. *Nat. Neurosci.*, *15*, 1460–1465.
- Aserinsky, E., & Kleitman, N. (1953). Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. *Science*, *118*, 273–274.
- Askitopoulou, H. (2015). Sleep and dreams: From myth to medicine in ancient greece. *J. Anesth. Hist.*, *1*, 70–75.
- Aston-Jones, G., & Bloom, F. E. (1981). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J. Neurosci.*, *1*, 876–886.
- Atienza, M., Cantero, J. L., & Escera, C. (2001). Auditory information processing during human sleep as revealed by event-related brain potentials. *Clin. Neurophysiol.*, *112*, 2031–2045.
- Bajo, V. M., & King, A. J. (2012). Cortical modulation of auditory processing in the midbrain. *Front. Neural Circuits*, *6*, 114.
- Baker, G. B., Coutts, R. T., McKenna, K. F., & Sherry-McKenna, R. L. (1992). Insights into the mechanisms of action of the MAO inhibitors phenelzine and tranylcypromine: A review. *J. Psychiatry Neurosci.*, *17*, 206–214.
- Barbanoj, M. J., Riba, J., Clos, S., Giménez, S., Grasa, E., & Romero, S. (2008). Daytime ayahuasca administration modulates REM and slow-wave sleep in healthy volunteers. *Psychopharmacology (Berl.)*, *196*, 315–326.

- Barrett, F. S., Doss, M. K., Sepeda, N. D., Pekar, J. J., & Griffiths, R. R. (2020). Emotions and brain function are altered up to one month after a single high dose of psilocybin. *Sci. Rep.*, *10*.
- Barrett, F. S., Johnson, M. W., & Griffiths, R. R. (2015). Validation of the revised mystical experience questionnaire in experimental sessions with psilocybin. *J. Psychopharmacol.*, *29*, 1182.
- Barsuglia, J., Davis, A. K., Palmer, R., Lancelotta, R., Windham-Herman, A. M., Peterson, K., Polanco, M., Grant, R., & Griffiths, R. R. (2018). Intensity of mystical experiences occasioned by 5-MeO-DMT and comparison with a prior psilocybin study. *Front. Psychol.*, *9*.
- Bartram, J., Kahn, M. C., Tuohy, S., Paulsen, O., Wilson, T., & Mann, E. O. (2017). Cortical up states induce the selective weakening of subthreshold synaptic inputs. *Nat. Commun.*, *8*, 665.
- Bassetti, C., McNicholas, W., Paunio, T., & Peigneux, P. (2022). *Sleep medicine textbook* (2nd ed.). European Sleep Research Society.
- Beck, L. H., Bransome, E. D., Jr, Mirsky, A. F., Rosvold, H. E., & Sarason, I. (1956). A continuous performance test of brain damage. *J. Consult. Psychol.*, *20*, 343–350.
- Benington, J. H., & Heller, H. C. (1995). Restoration of brain energy metabolism as the function of sleep. *Prog. Neurobiol.*, *45*, 347–360.
- Benington, J. H., & Heller, H. C. (1994). Does the function of REM sleep concern non-REM sleep or waking? *Prog. Neurobiol.*, *44*, 433–449.
- Bernardi, G., Betta, M., Ricciardi, E., Pietrini, P., Tononi, G., & Siclari, F. (2019). Regional delta waves in human rapid eye movement sleep. *J. Neurosci.*, *39*, 2686–2697.
- Blake, H., & Gerard, R. W. (1937). Brain potentials during sleep. *Am. J. Physiol.*, *119*, 692–703.
- Blake, H., Gerard, R. W., & Kleitman, N. (1938). Factors influencing brain potentials during sleep. *J. Neurophysiol.*, *2*, 48–60.
- Blumberg, M. S., Lesku, J. A., Libourel, P. A., Schmidt, M. H., & Rattenborg, N. C. (2020). What is REM sleep? *Curr. Biol.*, *30*, R38–R49.
- Blume, C., Del Giudice, R., Lechinger, J., Wislowska, M., Heib, D. P. J., Hoedlmoser, K., & Schabus, M. (2017). Preferential processing of emotionally and self-relevant stimuli persists in unconscious N2 sleep. *Brain Lang.*, *167*, 72–82.
- Bodizs, R. (2021, September 10). Theories on the functions of sleep. In C. Bassetti, W. McNicholas, T. Paunio, & P. Peigneux (Eds.), *Sleep medicine textbook* (2nd ed., pp. 41–56). European Sleep Research Society.
- Boland, E. M., Rao, H., Dinges, D. F., Smith, R. V., Goel, N., Detre, J. A., Basner, M., Sheline, Y. I., Thase, M. E., & Gehrman, P. R. (2017). Meta-analysis of the antidepressant effects of acute sleep deprivation. *J. Clin. Psychiatry*, *78*, e1020–e1034.
- Bonnet, M. H., Johnson, L. C., & Webb, W. B. (1978). The reliability of arousal threshold during sleep. *Psychophysiology*, *15*, 412–416.
- Boothman, L. J., Mitchell, S. N., & Sharp, T. (2006). Investigation of the SSRI augmentation properties of 5-HT(2) receptor antagonists using in vivo microdialysis. *Neuropharmacology*, *50*, 726–732.
- Borbély, A. A. (1982). A two process model of sleep regulation. *Hum. Neurobiol.*, *1*, 195–204.

- Borbély, A. A. (2022). The two-process model of sleep regulation: Beginnings and outlook. *J. Sleep Res.*, *31*, e13598.
- Borbély, A. A., Daan, S., Wirz-Justice, A., & Deboer, T. (2016). The two-process model of sleep regulation: A reappraisal. *J. Sleep Res.*, *25*, 131–143.
- Borbély, A. A., & Neuhaus, H. U. (1979). Sleep-deprivation: Effects on sleep and EEG in the rat. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.*, *133*, 71–87.
- Borbély, A. A., Tobler, I., & Hanagasioglu, M. (1984). Effect of sleep deprivation on sleep and EEG power spectra in the rat. *Behav. Brain Res.*, *14*, 171–182.
- Boyce, R., Glasgow, S. D., Williams, S., & Adamantidis, A. (2016). Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation. *Science*, *352*, 812–816.
- Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology*, *45*, 602–607.
- Bravermanová, A., Viktorinová, M., Tylš, F., Novák, T., Androvičová, R., Korčák, J., Horáček, J., Balíková, M., Griškova-Bulanova, I., Danielová, D., Vlček, P., Mohr, P., Brunovský, M., Koudelka, V., & Páleníček, T. (2018). Psilocybin disrupts sensory and higher order cognitive processing but not pre-attentive cognitive processing-study on P300 and mismatch negativity in healthy volunteers. *Psychopharmacology (Berl.)*, *235*, 491–503.
- Bremer, F. (1935). Cerveau isolé et physiologie du sommeil. *C. R. Seances Soc. Biol. Fil.*, *118*, 1235–1241.
- Bremer, F. (1936). Activité électrique du cortex cerebral dans les états de sommeil et de veille chez le chat. *Comptes rendus des seances de la Societe de biologie et de ses filiales*, *88*, 465–467.
- Brouwer, A., & Carhart-Harris, R. L. (2021). Pivotal mental states. *J. Psychopharmacol.*, *35*, 319–352.
- Brugge, J. F., Reale, R. A., & Hind, J. E. (1996). The structure of spatial receptive fields of neurons in primary auditory cortex of the cat. *J. Neurosci.*, *16*, 4420–4437.
- Bruhn, J. G., De Smet, P. A. G. M., El-Seedi, H. R., & Beck, O. (2002). Mescaline use for 5700 years. *Lancet*, *359*, 1866.
- Bunin, M. A., & Wightman, R. M. (1998). Quantitative evaluation of 5-hydroxytryptamine (serotonin) neuronal release and uptake: An investigation of extrasynaptic transmission. *J. Neurosci.*, *18*, 4854–4860.
- Buzsáki, G. (1998). Memory consolidation during sleep: A neurophysiological perspective. *J. Sleep Res.*, *7 Suppl 1*, 17–23.
- Buzsáki, G., Haubenreiser, J., Grastyán, E., Czopf, J., & Kellényi, L. (1981). Hippocampal slow wave activity during appetitive and aversive conditioning in the cat. *Electroencephalogr. Clin. Neurophysiol.*, *51*, 276–290.
- Buzsáki, G., Anastassiou, C. A., & Koch, C. (2012). The origin of extracellular fields and currents—EEG, ECoG, LFP and spikes. *Nat. Rev. Neurosci.*, *13*, 407–420.
- Cabrera, Y., Koymans, K. J., Poe, G. R., Kessels, H. W., Van Someren, E. J. W., & Wassing, R. (2024). Overnight neuronal plasticity and adaptation to emotional distress. *Nat. Rev. Neurosci.*, *25*, 253–271.
- Cameron, L. P., Tombari, R. J., Lu, J., Pell, A. J., Hurley, Z. Q., Ehinger, Y., Vargas, M. V., McCarroll, M. N., Taylor, J. C., Myers-Turnbull, D., Liu, T., Yaghoobi, B., Laskowski, L. J., Anderson, E. I., Zhang, G., Viswanathan, J., Brown, B. M., Tjia, M., Dunlap, L. E., . . . Olson, D. E. (2021). A

- non-hallucinogenic psychedelic analogue with therapeutic potential. *Nature*, 589, 474–479.
- Campbell, S. S., & Tobler, I. (1984). Animal sleep: A review of sleep duration across phylogeny. *Neurosci. Biobehav. Rev.*, 8, 269–300.
- Carhart-Harris, R. L., Bolstridge, M., Day, C. M. J., Rucker, J., Watts, R., Erritzoe, D. E., Kaelen, M., Giribaldi, B., Bloomfield, M., Pilling, S., Rickard, J. A., Forbes, B., Feilding, A., Taylor, D., Curran, H. V., & Nutt, D. J. (2018a). Psilocybin with psychological support for treatment-resistant depression: Six-month follow-up. *Psychopharmacology*, 235, 399–408.
- Carhart-Harris, R. L., Bolstridge, M., Rucker, J., Day, C. M. J., Erritzoe, D., Kaelen, M., Bloomfield, M., Rickard, J. A., Forbes, B., Feilding, A., Taylor, D., Pilling, S., Curran, V. H., & Nutt, D. J. (2016a). Psilocybin with psychological support for treatment-resistant depression: An open-label feasibility study. *The Lancet Psychiatry*, 3, 619–627.
- Carhart-Harris, R. L., & Goodwin, G. M. (2017). The therapeutic potential of psychedelic drugs: Past, present, and future. *Neuropsychopharmacology*, 42, 2105–2113.
- Carhart-Harris, R. L., Kaelen, M., Bolstridge, M., Williams, T. M., Williams, L. T., Underwood, R., Feilding, A., & Nutt, D. J. (2016b). The paradoxical psychological effects of lysergic acid diethylamide (LSD). *Psychol. Med.*, 46, 1379–1390.
- Carhart-Harris, R. L., Roseman, L., Haijen, E., Erritzoe, D., Watts, R., Branchi, I., & Kaelen, M. (2018b). Psychedelics and the essential importance of context. *J. Psychopharmacol.*, 32, 725–731.
- Carli, M., Robbins, T. W., Evenden, J. L., & Everitt, B. J. (1983). Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. *Behav. Brain Res.*, 9, 361–380.
- Carli, M., & Samanin, R. (1992). Serotonin<sub>2</sub> receptor agonists and serotonergic anorectic drugs affect rats' performance differently in a five-choice serial reaction time task. *Psychopharmacology (Berl.)*, 106, 228–234.
- Carlson, N. R. (2016, August 2). Sleep and biological rhythms. In N. R. Carlson & M. A. Birkett (Eds.), *Physiology of behavior, global edition*. Pearson Education.
- Carro-Domínguez, M., Huwiler, S., Oberlin, S., Oesch, T. L., Badii, G., Lüthi, A., Wenderoth, N., Meissner, S. N., & Lustenberger, C. (2023). Pupil size reveals arousal level dynamics in human sleep. *bioRxiv*, 2023.07.19.549720.
- Carter, O. L., Burr, D. C., Pettigrew, J. D., Wallis, G. M., Hasler, F., & Vollenweider, F. X. (2005). Using psilocybin to investigate the relationship between attention, working memory, and the serotonin 1A and 2A receptors. *J. Cogn. Neurosci.*, 17, 1497–1508.
- Carter, O. L., Hasler, F., Pettigrew, J. D., Wallis, G. M., Liu, G. B., & Vollenweider, F. X. (2007). Psilocybin links binocular rivalry switch rate to attention and subjective arousal levels in humans. *Psychopharmacology (Berl.)*, 195, 415–424.
- Castelnovo, A., Lopez, R., Proserpio, P., Nobili, L., & Dauvilliers, Y. (2018). NREM sleep parasomnias as disorders of sleep-state dissociation. *Nat. Rev. Neurol.*, 14, 470–481.
- Castro, E., Díaz, A., Rodríguez-Gaztelumendi, A., Del Olmo, E., & Pazos, A. (2008). WAY100635 prevents the changes induced by fluoxetine upon the 5-HT<sub>1A</sub> receptor functionality. *Neuropharmacology*, 55, 1391–1396.

- Cazettes, F., Reato, D., Morais, J. P., Renart, A., & Mainen, Z. F. (2021). Phasic activation of dorsal raphe serotonergic neurons increases pupil size. *Curr. Biol.*, *31*, 192–197.e4.
- Chandler, A. L. (1960). Lysergic acid diethylamide (LSD-25) as a facilitating agent in psychotherapy. *Arch. Gen. Psychiatry*, *2*, 286.
- Chemel, B. R., Roth, B. L., Armbruster, B., Watts, V. J., & Nichols, D. E. (2006). WAY-100635 is a potent dopamine D4 receptor agonist. *Psychopharmacology (Berl.)*, *188*, 244–251.
- Cirelli, C., & Tononi, G. (2008). Is sleep essential? *PLoS Biol.*, *6*, e216.
- Clément, O., Sapin, E., Bérød, A., Fort, P., & Luppi, P.-H. (2011). Evidence that neurons of the sublaterodorsal tegmental nucleus triggering paradoxical (REM) sleep are glutamatergic. *Sleep*, *34*, 419–423.
- Collins, D. R., Pelletier, J. G., & Paré, D. (2001). Slow and fast (gamma) neuronal oscillations in the perirhinal cortex and lateral amygdala. *J. Neurophysiol.*, *85*, 1661–1672.
- Connolly, P. M., Maxwell, C., Liang, Y., Kahn, J. B., Kanes, S. J., Abel, T., Gur, R. E., Turetsky, B. I., & Siegel, S. J. (2004). The effects of ketamine vary among inbred mouse strains and mimic schizophrenia for the P80, but not P20 or N40 auditory ERP components. *Neurochem. Res.*, *29*, 1179–1188.
- Cornea-Hébert, V., Riad, M., Wu, C., Singh, S. K., & Descarries, L. (1999). Cellular and subcellular distribution of the serotonin 5-HT<sub>2A</sub> receptor in the central nervous system of adult rat. *J. Comp. Neurol.*, *409*, 187–209.
- Crochet, S., & Sakai, K. (1999). Alpha-2 adrenoceptor mediated paradoxical (REM) sleep inhibition in the cat. *Neuroreport*, *10*, 2199–2204.
- Daan, S., Beersma, D. G., & Borbély, A. A. (1984). Timing of human sleep: Recovery process gated by a circadian pacemaker. *Am. J. Physiol.*, *246*, R161–83.
- Dang-Vu, T. T., McKinney, S. M., Buxton, O. M., Solet, J. M., & Ellenbogen, J. M. (2010). Spontaneous brain rhythms predict sleep stability in the face of noise. *Curr. Biol.*, *20*, R626–7.
- Darmani, N. A. (1998). The silent and selective 5-HT<sub>1A</sub> antagonist, WAY 100635, produces via an indirect mechanism, a 5-HT<sub>2A</sub> receptor-mediated behaviour in mice during the day but not at night. short communication. *J. Neural Transm. (Vienna)*, *105*, 635–643.
- David, F., Schmiedt, J. T., Taylor, H. L., Orban, G., Di Giovanni, G., Uebele, V. N., Renger, J. J., Lambert, R. C., Leresche, N., & Crunelli, V. (2013). Essential thalamic contribution to slow waves of natural sleep. *J. Neurosci.*, *33*, 19599–19610.
- Davis, C. J., Clinton, J. M., Jewett, K. A., Zielinski, M. R., & Krueger, J. M. (2011). Delta wave power: An independent sleep phenotype or epiphenomenon? *J. Clin. Sleep Med.*, *7*, S16.
- Davis, H., Davis, P. A., Loomis, A. L., Harvey, E. N., & Hobart, G. (1937). Changes in human brain potentials during the onset of sleep. *Science*, *86*, 448–450.
- de Vivo, L., Bellesi, M., Marshall, W., Bushong, E. A., Ellisman, M. H., Tononi, G., & Cirelli, C. (2017). Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. *Science*, *355*, 507–510.
- Deliens, G., Gilson, M., & Peigneux, P. (2014). Sleep and the processing of emotions. *Exp. Brain Res.*, *232*, 1403–1414.

- Deliens, G., Neu, D., & Peigneux, P. (2013). Rapid eye movement sleep does not seem to unbind memories from their emotional context. *J. Sleep Res.*, *22*, 656–662.
- Dement, W. (1958). The occurrence of low voltage, fast, electroencephalogram patterns during behavioral sleep in the cat. *Electroencephalogr. Clin. Neurophysiol.*, *10*, 291–296.
- Denber, H. C., & Merlis, S. (1955). Studies on mescaline. I. action in schizophrenic patients; clinical observations and brain wave patterns, showing effects before and after electric convulsive treatments. *Psychiatr. Q.*, *29*, 421–429.
- Destexhe, A., Contreras, D., & Steriade, M. M. (1999). Spatiotemporal analysis of local field potentials and unit discharges in cat cerebral cortex during natural wake and sleep states. *J. Neurosci.*, *19*, 4595–4608.
- Dinges, D. F., Pack, F., Williams, K., Gillen, K. A., Powell, J. W., Ott, G. E., Aptowicz, C., & Pack, A. I. (1997). Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. *Sleep*, *20*, 267–277.
- Doghramji, K., & Jangro, W. C. (2016). Adverse effects of psychotropic medications on sleep. *Psychiatr. Clin. North Am.*, *39*, 487–502.
- Domenico, C., Haggerty, D., Mou, X., & Ji, D. (2021). LSD degrades hippocampal spatial representations and suppresses hippocampal-visual cortical interactions. *Cell Rep.*, *36*, 109714.
- Du, Y., Li, Y., Zhao, X., Yao, Y., Wang, B., Zhang, L., & Wang, G. (2023). Psilocybin facilitates fear extinction in mice by promoting hippocampal neuroplasticity. *Chin. Med. J. (Engl.)*, *136*, 2983–2992.
- Dudysová, D., Janků, K., Šmotek, M., Saifutdinova, E., Kopřivová, J., Bušková, J., Mander, B. A., Brunovský, M., Zach, P., Korčák, J., Andrashko, V., Viktorinová, M., Tylš, F., Bravermanová, A., Froese, T., Páleníček, T., & Horáček, J. (2020). The effects of daytime psilocybin administration on sleep: Implications for antidepressant action. *Front. Pharmacol.*, *11*, 602590.
- Duncan, W. C., Jr, Ballard, E. D., & Zarate, C. A. (2019). Ketamine-induced glutamatergic mechanisms of sleep and wakefulness: Insights for developing novel treatments for disturbed sleep and mood. *Handb. Exp. Pharmacol.*, *253*, 337–358.
- Duncan, W. C., Sarasso, S., Ferrarelli, F., Selter, J., Riedner, B. A., Hejazi, N. S., Yuan, P., Brutsche, N., Manji, H. K., Tononi, G., & Zarate, C. A. (2013). Concomitant BDNF and sleep slow wave changes indicate ketamine-induced plasticity in major depressive disorder. *Int. J. Neuropsychopharmacol.*, *16*, 301–311.
- Eban-Rothschild, A., Rothschild, G., Giardino, W. J., Jones, J. R., & de Lecea, L. (2016). VTA dopaminergic neurons regulate ethologically relevant sleep-wake behaviors. *Nat. Neurosci.*, *19*, 1356–1366.
- Ebitz, R. B., Pearson, J. M., & Platt, M. L. (2014). Pupil size and social vigilance in rhesus macaques. *Front. Neurosci.*, *8*, 100.
- Ehrlichman, R. S., Maxwell, C. R., Majumdar, S., & Siegel, S. J. (2008). Deviance-elicited changes in event-related potentials are attenuated by ketamine in mice. *J. Cogn. Neurosci.*, *20*, 1403–1414.
- Ekirch, A. R. (2016). Segmented sleep in preindustrial societies. *Sleep*, *39*, 715–716.
- Ermakova, A. O., Dunbar, F., Rucker, J., & Johnson, M. W. (2022). A narrative synthesis of research with 5-MeO-DMT. *J. Psychopharmacol.*, *36*, 273–294.

- Evenden, J. L. (1999). Varieties of impulsivity. *Psychopharmacology (Berl.)*, 146, 348–361.
- Fernandez, L. M. J., & Lüthi, A. (2020). Sleep spindles: Mechanisms and functions. *Physiol. Rev.*, 100, 805–868.
- Fischer, R., Hill, R., Thatcher, K., & Scheib, J. (1970). Psilocybin-induced contraction of nearby visual space. *Agents Actions*, 1, 190–197.
- Fisher, S. P., Cui, N., McKillop, L. E., Gemignani, J., Bannerman, D. M., Oliver, P. L., Peirson, S. N., & Vyazovskiy, V. V. (2016). Stereotypic wheel running decreases cortical activity in mice. *Nat. Commun.*, 7.
- Flood, M. D., & Eggers, E. D. (2021). Dopamine D1 and D4 receptors contribute to light adaptation in ON-sustained retinal ganglion cells. *J. Neurophysiol.*, 126, 2039–2052.
- Forster, E. A., Cliffe, I. A., Bill, D. J., Dover, G. M., Jones, D., Reilly, Y., & Fletcher, A. (1995). A pharmacological profile of the selective silent 5-HT<sub>1A</sub> receptor antagonist, WAY-100635. *Eur. J. Pharmacol.*, 281, 81–88.
- Fort, P., Bassetti, C. L., & Luppi, P.-H. (2009). Alternating vigilance states: New insights regarding neuronal networks and mechanisms. *Eur. J. Neurosci.*, 29, 1741–1753.
- Fortier-Poisson, P., & Smith, A. M. (2016). Neuronal activity in somatosensory cortex related to tactile exploration. *J. Neurophysiol.*, 115, 112–126.
- Fox, M. A., Stein, A. R., French, H. T., & Murphy, D. L. (2010). Functional interactions between 5-HT<sub>2A</sub> and presynaptic 5-HT<sub>1A</sub> receptor-based responses in mice genetically deficient in the serotonin 5-HT transporter (SERT): 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> interactions in SERT-deficient mice. *Br. J. Pharmacol.*, 159, 879–887.
- Frank, M. G., Issa, N. P., & Stryker, M. P. (2001). Sleep enhances plasticity in the developing visual cortex. *Neuron*, 30, 275–287.
- Franken, P. (2002). Long-term vs. short-term processes regulating REM sleep. *J. Sleep Res.*, 11, 17–28.
- Franken, P., Malafosse, A., & Tafti, M. (1998). Genetic variation in EEG activity during sleep in inbred mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 275, R1127–R1137.
- Frase, L., & Nissen, C. (2022). Substance use disorder. In C. Bassetti (Ed.), *Sleep medicine textbook* (2nd ed., pp. 855–862). European Sleep Research Society.
- Fu, V. X., Sleurink, K. J., Janssen, J. C., Wijnhoven, B. P. L., Jeekel, J., & Klimek, M. (2021). Perception de stimuli auditifs pendant l'anesthésie générale et ses effets sur les devenir des patients : Revue systématique et méta-analyse. [Perception of auditory stimuli during general anesthesia and its effects on patient outcomes: A systematic review and meta-analysis]. *Can. J. Anaesth.*, 68, 1231–1253.
- Funk, C. M., Honjoh, S., Rodriguez, A. V., Cirelli, C., & Tononi, G. (2016). Local slow waves in superficial layers of primary cortical areas during REM sleep. *Curr. Biol.*, 26, 396–403.
- Gavriloff, D., Kyle, S. D., & Espie, C. Q. (2022). Cognitive behavioural therapy for insomnia and other psychotherapeutic approaches. In C. Bassetti, W. McNicholas, T. Paunio, & P. Peigneux (Eds.), *Sleep medicine textbook* (2nd ed., pp. 265–278). European sleep research society.
- Gazzaniga, M. S., Ivry, R. B., & Mangun, G. R. (2009). *Cognitive neuroscience : The biology of the mind* (3rd ed.). W.W. Norton.
- Geoffroy, P. A., Hoertel, N., Etain, B., Bellivier, F., Delorme, R., Limosin, F., & Peyre, H. (2018). Insomnia and hypersomnia in major depressive episode:

- Prevalence, sociodemographic characteristics and psychiatric comorbidity in a population-based study. *J. Affect. Disord.*, 226, 132–141.
- Geyer, M. A., & Light, R. K. (1979). LSD-induced alterations of investigatory responding in rats. *Psychopharmacology (Berl.)*, 65, 41–47.
- Glass, J. D., Selim, M., & Rea, M. A. (1994). Modulation of light-induced C-fos expression in the suprachiasmatic nuclei by 5-HT<sub>1A</sub> receptor agonists. *Brain research*, 638(1-2).
- Gong, H., McGinty, D., Guzman-Marin, R., Chew, K.-T., Stewart, D., & Szymusiak, R. (2004). Activation of c-fos in GABAergic neurones in the preoptic area during sleep and in response to sleep deprivation. *J. Physiol.*, 556, 935–946.
- González-Maeso, J., Weisstaub, N. V., Zhou, M., Chan, P., Ivic, L., Ang, R., Lira, A., Bradley-Moore, M., Ge, Y., Zhou, Q., Sealton, S. C., & Gingrich, J. A. (2007). Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. *Neuron*, 53, 439–452.
- González-Rueda, A., Pedrosa, V., Feord, R. C., Clopath, C., & Paulsen, O. (2018). Activity-dependent downscaling of subthreshold synaptic inputs during slow-wave-sleep-like activity in vivo. *Neuron*, 97, 1244–1252.e5.
- Griffiths, R. R., Richards, W. A., McCann, U., & Jesse, R. (2006). Psilocybin can occasion mystical-type experiences having substantial and sustained personal meaning and spiritual significance. *Psychopharmacology*, 187, 268–283.
- Griffiths, R. R., Johnson, M. W., Carducci, M. A., Umbricht, A., Richards, W. A., Richards, B. D., Cosimano, M. P., & Klinedinst, M. A. (2016). Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: A randomized double-blind trial. *J Psychopharmacol*, 30, 1181–1197.
- Griffiths, R. R., Johnson, M. W., Richards, W. A., Richards, B. D., McCann, U., & Jesse, R. (2011). Psilocybin occasioned mystical-type experiences: Immediate and persisting dose-related effects. *Psychopharmacology (Berl.)*, 218, 649–665.
- Grosmark, A. D., Mizuseki, K., Pastalkova, E., Diba, K., & Buzsáki, G. (2012). REM sleep reorganizes hippocampal excitability. *Neuron*, 75, 1001–1007.
- Gross, C. G. (1998). Claude bernard and the constancy of the internal environment. *Neuroscientist*, 4, 380–385.
- Guttman, E. (1936). Artificial psychoses produced by mescaline. *J. Ment. Sci.*, 82, 203–221.
- Haberzettl, R., Bert, B., Fink, H., & Fox, M. A. (2013). Animal models of the serotonin syndrome: A systematic review. *Behav. Brain Res.*, 256, 328–345.
- Hagan, J. J., Price, G. W., Jeffrey, P., Deeks, N. J., Stean, T., Piper, D., Smith, M. I., Upton, N., Medhurst, A. D., Middlemiss, D. N., Riley, G. J., Lovell, P. J., Bromidge, S. M., & Thomas, D. R. (2000). Characterization of SB-269970-a, a selective 5-HT(7) receptor antagonist: SB-269970-a, a selective 5-HT<sub>7</sub>receptor antagonist. *Br. J. Pharmacol.*, 130, 539–548.
- Halberstadt, A. L., & Geyer, M. A. (2010). Hallucinogens. In *Encyclopedia of behavioral neuroscience* (pp. 12–20). Elsevier.
- Halberstadt, A. L. (2015). Recent advances in the neuropsychopharmacology of serotonergic hallucinogens. *Behav. Brain Res.*, 277, 99–120.
- Halberstadt, A. L., Chatha, M., Klein, A. K., Wallach, J., & Brandt, S. D. (2020). Correlation between the potency of hallucinogens in the mouse head-twitch



- response assay and their behavioral and subjective effects in other species. *Neuropharmacology*, 167, 107933.
- Halberstadt, A. L., & Geyer, M. A. (2011). Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. *Neuropharmacology*, 61(3), 364–381.
- Halberstadt, A. L., Koedood, L., Powell, S. B., & Geyer, M. A. (2011). Differential contributions of serotonin receptors to the behavioral effects of indoleamine hallucinogens in mice. *J. Psychopharmacol.*, 25.
- Halberstadt, A. L., Nichols, D. E., & Geyer, M. A. (2012). Behavioral effects of  $\alpha,\alpha,\beta,\beta$ -tetrahydro-5-MeO-DMT in rats: Comparison with 5-MeO-DMT administered in combination with a monoamine oxidase inhibitor. *Psychopharmacology*, 221, 709–718.
- Hanks, J. B., & González-Maeso, J. (2013). Animal models of serotonergic psychedelics. *ACS Chem. Neurosci.*, 4, 33–42.
- Harding, C. (2022). *Sleep and torpor in rodents and bats: From brain activity to social interactions* [Doctoral dissertation, University of Oxford].
- Harrison, A. A., Everitt, B. J., & Robbins, T. W. (1997a). Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: Interactions with dopaminergic mechanisms. *Psychopharmacology (Berl.)*, 133, 329–342.
- Harrison, A. A., Everitt, B. J., & Robbins, T. W. (1997b). Doubly dissociable effects of median- and dorsal-raphé lesions on the performance of the five-choice serial reaction time test of attention in rats. *Behav. Brain Res.*, 89, 135–149.
- Hauner, K. K., Howard, J. D., Zelano, C., & Gottfried, J. A. (2013). Stimulus-specific enhancement of fear extinction during slow-wave sleep. *Nat. Neurosci.*, 16, 1553–1555.
- Hedlund, P. B., Huitron-Resendiz, S., Henriksen, S. J., & Sutcliffe, J. G. (2005). 5-HT7 receptor inhibition and inactivation induce antidepressantlike behavior and sleep pattern. *Biol. Psychiatry*, 58, 831–837.
- Hermle, L., Gouzoulis-Mayfrank, E., & Spitzer, M. (1998). Blood flow and cerebral laterality in the mescaline model of psychosis. *Pharmacopsychiatry*, 31 Suppl 2, 85–91.
- Hernandez-Peon, R., Scherrer, H., & Jouvet, M. (1956). Modification of electric activity in cochlear nucleus during attention in unanesthetized cats. *Science*, 123, 331–332.
- Hess, R., Koella, W. P., & Akert, K. (1953). Cortical and subcortical recordings in natural and artificially induced sleep in cats. *Electroencephalogr. Clin. Neurophysiol.*, 5, 75–90.
- Hinard, V., Mikhail, C., Pradervand, S., Curie, T., Houtkooper, R. H., Auwerx, J., Franken, P., & Tafti, M. (2012). Key electrophysiological, molecular, and metabolic signatures of sleep and wakefulness revealed in primary cortical cultures. *J. Neurosci.*, 32, 12506–12517.
- Hobson, J. A. (1988, June 20). *The dreaming brain*. Basic Books.
- Hoch, P. H., Cattell, J. P., & Pennes, H. H. (1952). Effects of mescaline and lysergic acid (d-LSD-25). *Am. J. Psychiatry*, 108, 579–584.
- Hofmann, A., & Feilding, A. (2013, April 9). *LSD: My problem child* (J. Ott, Trans.). Oxford University Press.
- Huber, R., Tononi, G., & Cirelli, C. (2007). Exploratory behavior, cortical BDNF expression, and sleep homeostasis. *Sleep*, 30, 129–139.

- Hubl, D., Koenig, T., Strik, W. K., Garcia, L. M., & Dierks, T. (2007). Competition for neuronal resources: How hallucinations make themselves heard. *Br. J. Psychiatry*, *190*, 57–62.
- Hughes, S., Pothecary, C. A., Jagannath, A., Foster, R. G., Hankins, M. W., & Peirson, S. N. (2012). Profound defects in pupillary responses to light in TRPM-channel null mice: A role for TRPM channels in non-image-forming photoreception: A role for TRPM in non-image-forming responses to light. *Eur. J. Neurosci.*, *35*, 34–43.
- Husain, A. M. (2006). Electroencephalographic assessment of coma. *J. Clin. Neurophysiol.*, *23*, 208–220.
- Idir, Y., Oudiette, D., & Arnulf, I. (2022). Sleepwalking, sleep terrors, sexsomnia and other disorders of arousal: The old and the new. *J. Sleep Res.*, e13596.
- Ison, J. R., & Allen, P. D. (2003). Low-frequency tone pips elicit exaggerated startle reflexes in C57BL/6J mice with hearing loss. *J. Assoc. Res. Otolaryngol.*, *4*, 495–504.
- Jakab, R. L., & Goldman-Rakic, P. S. (1998). 5-Hydroxytryptamine<sub>2A</sub> serotonin receptors in the primate cerebral cortex: Possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. *Proc. Natl. Acad. Sci. U. S. A.*, *95*, 735–740.
- Jenkins, J. G., & Dallenbach, K. M. (1924). Obliviscence during sleep and waking. *Am. J. Psychol.*, *35*, 605.
- Joshi, S., & Gold, J. I. (2020). Pupil size as a window on neural substrates of cognition. *Trends Cogn. Sci.*, *24*, 466–480.
- Jouvet, D., Vimont, P., Delorme, F., & Jouvet, M. (1964). Etude de la privation sélective de la phase paradoxale de sommeil chez le chat. [Study of selective deprivation of the paradoxal sleep phase in the cat]. *C. R. Seances Soc. Biol. Fil.*, *158*, 756–759.
- Jouvet, M. (1999). Sleep and serotonin an unfinished story. *Neuropsychopharmacology*, *21*, 24S–27S.
- Jouvet, M., Lapras, C., & Hermann, A. (1959a). Variations des réponses électriques somesthésiques au niveau du thalamus chez l'homme au cours de l'attention. [Changes in somesthetic electric responses at the level of the thalamus in humans during attention to the stimulus]. *C. R. Seances Soc. Biol. Fil.*, *153*, 98–101.
- Jouvet, M., & Michel, F. (1958). Recherches sur l'activité électrique cérébrale au cours du sommeil. [Study of the cerebral electrical activity during sleep]. *C. R. Seances Soc. Biol. Fil.*, *152*, 1167–1170.
- Jouvet, M., & Michel, F. (1959). Corrélations électromyographique du sommeil chez le chat décortiqué et mésencéphalique chronique. [Electromyographic correlations of sleep in the chronic decorticate & mesencephalic cat]. *C. R. Seances Soc. Biol. Fil.*, *153*, 422–425.
- Jouvet, M., Michel, F., & Courjon, J. (1959b). L'activité électrique du rhinencéphale au cours du sommeil chez le chat. [Electric activity of the rhinencephalon during sleep in cats]. *C. R. Seances Soc. Biol. Fil.*, *153*, 101–105.
- Jouvet, M., Michel, F., & Courjon, J. (1959c). On a stage of rapid cerebral electrical activity in the course of physiological sleep. *C. R. Seances Soc. Biol. Fil.*, *153*, 1024–1028.
- Kahn, M., Krone, L. B., Blanco-Duque, C., Guillaumin, M. C. C., Mann, E. O., & Vyazovskiy, V. V. (2022). Neuronal-spiking-based closed-loop stimulation during cortical ON- and OFF-states in freely moving mice. *J. Sleep Res.*, *31*.

- Kaiser, W., & Steiner-Kaiser, J. (1983). Neuronal correlates of sleep, wakefulness and arousal in a diurnal insect. *Nature*, *301*, 707–709.
- Kattler, H., Dijk, D. J., & Borbély, A. A. (1994). Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. *J. Sleep Res.*, *3*, 159–164.
- Klein, M., Michel, F., & Jouvet, M. (1964). Etude polygraphique du sommeil chez les oiseaux. [Polygraphic study of sleep in birds]. *C. R. Seances Soc. Biol. Fil.*, *158*, 99–103.
- Kometer, M., Schmidt, A., Bachmann, R., Studerus, E., Seifritz, E., & Vollenweider, F. X. (2012). Psilocybin biases facial recognition, goal-directed behavior, and mood state toward positive relative to negative emotions through different serotonergic subreceptors. *Biol. Psychiatry*, *72*, 898–906.
- Koskinen, T., Ruotsalainen, S., Puumala, T., Lappalainen, R., Koivisto, E., Männistö, P. T., & Sirviö, J. (2000a). Activation of 5-HT<sub>2A</sub> receptors impairs response control of rats in a five-choice serial reaction time task. *Neuropharmacology*, *39*, 471–481.
- Koskinen, T., Ruotsalainen, S., & Sirviö, J. (2000b). The 5-HT<sub>2</sub> receptor activation enhances impulsive responding without increasing motor activity in rats. *Pharmacol. Biochem. Behav.*, *66*, 729–738.
- Kouider, S., Andrillon, T., Barbosa, L. S., Goupil, L., & Bekinschtein, T. A. (2014). Inducing task-relevant responses to speech in the sleeping brain. *Curr. Biol.*, *24*, 2208–2214.
- Krone, L. B. (2023, April). *Cortical regulation of sleep* [Doctoral dissertation, University of Oxford].
- Krone, L. B., & Vyazovskiy, V. V. (2020). Unresponsive or just asleep? do local slow waves in the perilesional cortex have a function? *Brain*, *143*, 3513–3515.
- Krone, L. B., Yamagata, T., Blanco-Duque, C., Guillemin, M. C. C., Kahn, M. C., van der Vinne, V., McKillop, L. E., Tam, S. K. E., Peirson, S. N., Akerman, C. J., Hoerder-Suabedissen, A., Molnár, Z., & Vyazovskiy, V. V. (2021). A role for the cortex in sleep-wake regulation. *Nat. Neurosci.*, *24*, 1210–1215.
- Kwan, A. C., Olson, D. E., Preller, K. H., & Roth, B. L. (2022). The neural basis of psychedelic action. *Nat. Neurosci.*, *25*, 1407–1419.
- Landolt, H.-P. (2012). "no thanks, coffee keeps me awake": Individual caffeine sensitivity depends on ADORA2A genotype. *Sleep*, *35*, 899–900.
- Larsen, J. K. (2016). Neurotoxicity and LSD treatment: A follow-up study of 151 patients in denmark. *Hist. Psychiatry*, *27*, 172–189.
- Larsen, R. S., & Waters, J. (2018). Neuromodulatory correlates of pupil dilation. *Front. Neural Circuits*, *12*, 21.
- Lee, E. E., Della Selva, M. P., Liu, A., & Himelhoch, S. (2015). Ketamine as a novel treatment for major depressive disorder and bipolar depression: A systematic review and quantitative meta-analysis. *Gen. Hosp. Psychiatry*, *37*, 178–184.
- Lee, M. A., & Shlain, B. (2007, December 1). *Acid dreams: The complete social history of LSD: The cia, the sixties, and beyond*. Grove Press.
- Leonard, J. A. (1959). Five-choice serial reaction apparatus. *Med. Res. Coun. Appl. Psychol. Res. Unit.*, *326*.
- Lepow, L., Kronman, H., & Yehuda, R. (2023). A heuristic approach: Against consensus definitions of psychedelics. *Psychedelic Medicine*, *1*, 190–194.
- Letcher, A. (2007). Shroom: A cultural history of the magic mushroom.

- Liddell, D. W., & Weil-Malherbe, H. (1953). The effects of methedrine and of lysergic acid diethylamide on mental processes and on the blood adrenaline level. *J. Neurol. Neurosurg. Psychiatry*, *16*, 7–13.
- Loomis, A. L., Harvey, E. N., & Hobart, G. (1935). Further observations on the potential rhythms of the cerebral cortex during sleep. *Science*, *82*, 198–200.
- Loomis, A. L., Harvey, E. N., & Hobart, G. (1936). Brain potentials during hypnosis. *Science*, *83*, 239–241.
- Loomis, A. L., Harvey, E. N., & Hobart, G. A. (1937). Cerebral states during sleep, as studied by human brain potentials. *J. Exp. Psychol.*, *21*, 127–144.
- Luppi, P.-H., Adamantidis, A., & Fort, P. (2022). The neurophysiology and neurobiology of paradoxical (REM) sleep. In C. Bassetti, W. McNicholas, T. Paunio, & P. Peigneux (Eds.), *Sleep medicine textbook* (2nd ed., pp. 15–25). European Sleep Research Society.
- Lyamin, O. I., Kosenko, P. O., Korneva, S. M., Vyssotski, A. L., Mukhametov, L. M., & Siegel, J. M. (2018). Fur seals suppress REM sleep for very long periods without subsequent rebound. *Curr. Biol.*, *28*, 2000–2005.e2.
- Madan, V., & Jha, S. K. (2012). Sleep alterations in mammals: Did aquatic conditions inhibit rapid eye movement sleep? *Neurosci. Bull.*, *28*, 746–758.
- Madsen, M. K., Fisher, P. M., Burmester, D., Dyssegaard, A., Stenbæk, D. S., Kristiansen, S., Johansen, S. S., Lehel, S., Linnet, K., Svarer, C., Erritzoe, D., Ozenne, B., & Knudsen, G. M. (2019). Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels. *Neuropsychopharmacology*, *44*, 1328–1334.
- Magoun, H. W. (1950). Caudal and cephalic influences of the brain stem reticular formation. *Physiol. Rev.*, *30*, 459–474.
- Mar, A. C., Horner, A. E., Nilsson, S. R. O., Alsiö, J., Kent, B. A., Kim, C. H., Holmes, A., Saksida, L. M., & Bussey, T. J. (2013). The touchscreen operant platform for assessing executive function in rats and mice. *Nat. Protoc.*, *8*, 1985–2005.
- Marrosu, F., Portas, C., Mascia, M. S., Casu, M. A., Fà, M., Giagheddu, M., Imperato, A., & Gessa, G. L. (1995). Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats. *Brain Res.*, *671*, 329–332.
- Martin, P., Frances, H., & Simon, P. (1985). Dissociation of head twitches and tremors during the study of interactions with 5-hydroxytryptophan in mice. *J. Pharmacol. Methods*, *13*(3), 193–200.
- Martinez, D., Hwang, D., Mawlawi, O., Slifstein, M., Kent, J., Simpson, N., Parsey, R. V., Hashimoto, T., Huang, Y., Shinn, A., Van Heertum, R., Abi-Dargham, A., Caltabiano, S., Malizia, A., Cowley, H., Mann, J. J., & Laruelle, M. (2001). Differential occupancy of somatodendritic and postsynaptic 5HT(1A) receptors by pindolol: A dose-occupancy study with [<sup>11</sup>C]way 100635 and positron emission tomography in humans. *Neuropsychopharmacology*, *24*, 209–229.
- Mason, N. L., Kuypers, K. P. C., Müller, F., Reckweg, J., Tse, D. H. Y., Toennes, S. W., Hutten, N. R. P. W., Jansen, J. F. A., Stiers, P., Feilding, A., & Ramaekers, J. G. (2020). Me, myself, bye: Regional alterations in glutamate and the experience of ego dissolution with psilocybin. *Neuropsychopharmacology*, *45*, 2003–2011.

- Masson, J., Emerit, M. B., Hamon, M., & Darmon, M. (2012). Serotonergic signaling: Multiple effectors and pleiotropic effects. *Wiley Interdiscip. Rev. Membr. Transp. Signal.*, 1(6), 685–713.
- Mathis, A., Mamidanna, P., Cury, K. M., Abe, T., Murthy, V. N., Mathis, M. W., & Bethge, M. (2018). DeepLabCut: Markerless pose estimation of user-defined body parts with deep learning. *Nat. Neurosci.*, 21, 1281–1289.
- Maurer, J., Lin, A., Jin, X., Hong, J., Sathi, N., Cardis, R., Osorio-Forero, A., Lüthi, A., Weber, F., & Chung, S. (2024). Homeostatic regulation of REM sleep by the preoptic area of the hypothalamus. *eLife*.
- Maxwell, C. R., Liang, Y., Weightman, B. D., Kanes, S. J., Abel, T., Gur, R. E., Turetsky, B. I., Bilker, W. B., Lenox, R. H., & Siegel, S. J. (2004). Effects of chronic olanzapine and haloperidol differ on the mouse N1 auditory evoked potential. *Neuropsychopharmacology*, 29, 739–746.
- Maya Vetencourt, J. F., Sale, A., Viegi, A., Baroncelli, L., De Pasquale, R., O’Leary, O. F., Castrén, E., & Maffei, L. (2008). The antidepressant fluoxetine restores plasticity in the adult visual cortex. *Science*, 320, 385–388.
- Mazzola-Pomietto, P., Aulakh, C. S., Wozniak, K. M., Hill, J. L., & Murphy, D. L. (1995). Evidence that 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced hyperthermia in rats is mediated by stimulation of 5-HT<sub>2A</sub> receptors. *Psychopharmacology*, 117, 193–199.
- McCarthy, A., Wafford, K., Shanks, E., Ligocki, M., Edgar, D. M., & Dijk, D. J. (2016). REM sleep homeostasis in the absence of REM sleep: Effects of antidepressants. *Neuropharmacology*, 108, 415–425.
- McCormick, D. A., & Bal, T. (1994). Sensory gating mechanisms of the thalamus. *Curr. Opin. Neurobiol.*, 4, 550–556.
- McGinley, M. J., Vinck, M., Reimer, J., Batista-Brito, R., Zagha, E., Cadwell, C. R., Tolia, A. S., Cardin, J. A., & McCormick, D. A. (2015). Waking state: Rapid variations modulate neural and behavioral responses. *Neuron*, 87, 1143–1161.
- McKillop, L. E., Fisher, S. P., Cui, N., Peirson, S. N., Foster, R. G., Wafford, K. A., & Vyazovskiy, V. V. (2018). Effects of aging on cortical neural dynamics and local sleep homeostasis in mice. *J. Neurosci.*, 38, 3911.
- Meddis, R. (1977, September 10). *The sleep instinct*. Routledge.
- Meijer, J. H., Watanabe, K., Schaap, J., Albus, H., & Déttari, L. (1998). Light responsiveness of the suprachiasmatic nucleus: Long-term multiunit and single-unit recordings in freely moving rats. *J. Neurosci.*, 18, 9078–9087.
- Melo, M. C. A., Abreu, R. L. C., Linhares Neto, V. B., de Bruin, P. F. C., & de Bruin, V. M. S. (2017). Chronotype and circadian rhythm in bipolar disorder: A systematic review. *Sleep Med. Rev.*, 34, 46–58.
- Merlis, S. (1957). The effects of mescaline sulfate in chronic schizophrenia. *J. Nerv. Ment. Dis.*, 125, 432–434.
- Meyer, A. F., Poort, J., O’Keefe, J., Sahani, M., & Linden, J. F. (2018). A head-mounted camera system integrates detailed behavioral monitoring with multichannel electrophysiology in freely moving mice. *Neuron*, 100, 46–60.e7.
- Michaël, A. M., Abe, E. T., & Niell, C. M. (2020). Dynamics of gaze control during prey capture in freely moving mice. *eLife*, 9.
- Milinski, L., Fisher, S. P., Cui, N., McKillop, L. E., Blanco-Duque, C., Ang, G., Yamagata, T., Bannerman, D. M., & Vyazovskiy, V. V. (2021). Waking experience modulates sleep need in mice. *BMC Biol.*, 19, 65.

- Miner, L. A. H., Backstrom, J. R., Sanders-Bush, E., & Sesack, S. R. (2003). Ultrastructural localization of serotonin<sub>2A</sub> receptors in the middle layers of the rat prelimbic prefrontal cortex. *Neuroscience*, *116*, 107–117.
- Mirsky, A. F., & Rosvold, H. E. (1960). The use of psychoactive drugs as a neuropsychological tool in studies of attention in man. *Drugs and behavior*, *375–392*.
- Mitchell, S. J., Rawlins, J. N., Steward, O., & Olton, D. S. (1982). Medial septal area lesions disrupt theta rhythm and cholinergic staining in medial entorhinal cortex and produce impaired radial arm maze behavior in rats. *J. Neurosci.*, *2*, 292–302.
- Miyasaka, M., & Domino, E. F. (1968). Neural mechanisms of ketamine-induced anesthesia. *Int. J. Neuropharmacol.*, *7*, 557–573.
- Moliner, R., Girych, M., Brunello, C. A., Kovaleva, V., Biojone, C., Enkavi, G., Antenucci, L., Kot, E. F., Goncharuk, S. A., Kaurinkoski, K., Kuutti, M., Fred, S. M., Elsilä, L. V., Sakson, S., Cannarozzo, C., Diniz, C. R. A. F., Seiffert, N., Rubiolo, A., Haapaniemi, H., ... Castrén, E. (2023). Psychedelics promote plasticity by directly binding to BDNF receptor TrkB. *Nat. Neurosci.*, *26*, 1032–1041.
- Monti, J. M. (2011). Serotonin control of sleep-wake behavior. *Sleep Med. Rev.*, *15*, 269–281.
- Moore, R. Y., & Eichler, V. B. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Res.*, *42*, 201–206.
- Moore, R. Y., & Lenn, N. J. (1972). A retinohypothalamic projection in the rat. *J. Comp. Neurol.*, *146*, 1–14.
- Moruzzi, G., & Magoun, H. W. (1949). Brain stem reticular formation and activation of the EEG. *Electroencephalogr. Clin. Neurophysiol.*, *1*, 455–473.
- Moser, P. C., & Redfern, P. H. (1985). Circadian variation in behavioural responses to central 5-HT receptor stimulation in the mouse. *Psychopharmacology*, *86*, 223–227.
- Müller, C. P., & Cunningham, K. A. (2020). *Handbook of the behavioral neurobiology of serotonin* (2nd ed., Vol. 31). Academic Press.
- Muller-Gass, A., & Campbell, K. (2019). Sleep deprivation moderates neural processes associated with passive auditory capture. *Brain Cogn.*, *132*, 89–97.
- Murray, C. H., Tare, I., Perry, C. M., Malina, M., Lee, R., & de Wit, H. (2022). Low doses of LSD reduce broadband oscillatory power and modulate event-related potentials in healthy adults. *Psychopharmacology*, *239*, 1735–1747.
- Muzerelle, A., Scotto-Lomassese, S., Bernard, J. F., Soiza-Reilly, M., & Gaspar, P. (2016). Conditional anterograde tracing reveals distinct targeting of individual serotonin cell groups (B5-B9) to the forebrain and brainstem. *Brain Struct. Funct.*, *221*, 535–561.
- Nagai, F., Nonaka, R., & Satoh Hisashi Kamimura, K. (2007). The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. *Eur. J. Pharmacol.*, *559*, 132–137.
- Nakazawa, S., Nakamichi, K., Imai, H., & Ichihara, J. (2015). Effect of dopamine D4 receptor agonists on sleep architecture in rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *63*, 6–13.
- Nardou, R., Sawyer, E., Song, Y. J., Wilkinson, M., Padovan-Hernandez, Y., de Deus, J. L., Wright, N., Lama, C., Faltin, S., Goff, L. A., Stein-O'Brien, G. L.,

- & Dölen, G. (2023). Psychedelics reopen the social reward learning critical period. *Nature*, *618*, 790–798.
- Nath, T., Mathis, A., Chen, A. C., Patel, A., Bethge, M., & Mathis, M. W. (2019). Using DeepLabCut for 3D markerless pose estimation across species and behaviors. *Nat. Protoc.*, *14*, 2152–2176.
- Nauta, W. J. H. (1946). Hypothalamic regulation of sleep in rats; an experimental study. *J. Neurophysiol.*, *9*, 285–316.
- Nichols, D. E., & Nichols, C. D. (2008). Serotonin receptors. *Chem. Rev.*, *108*, 1614–1641.
- Nichols, D. E., Nichols, C. D., & Hendricks, P. S. (2022). Proposed consensus statement on defining psychedelic drugs. *Psychedelic Medicine*, *1*, 12–13.
- Nir, Y., Andrillon, T., Marmelshtein, A., Suthana, N., Cirelli, C., Tononi, G., & Fried, I. (2017). Selective neuronal lapses precede human cognitive lapses following sleep deprivation. *Nat. Med.*, *23*, 1474–1480.
- Nir, Y., Staba, R. J., Andrillon, T., Vyazovskiy, V. V., Cirelli, C., Fried, I., & Tononi, G. (2011). Regional slow waves and spindles in human sleep. *Neuron*, *70*, 153–169.
- Nir, Y., Vyazovskiy, V. V., Cirelli, C., Banks, M. I., & Tononi, G. (2015). Auditory responses and stimulus-specific adaptation in rat auditory cortex are preserved across NREM and REM sleep. *Cereb. Cortex*, *25*, 1362–1378.
- Norimoto, H., Fenk, L. A., Li, H.-H., Tosches, M. A., Gallego-Flores, T., Hain, D., Reiter, S., Kobayashi, R., Macias, A., Arends, A., Klinkmann, M., & Laurent, G. (2020). A claustrum in reptiles and its role in slow-wave sleep. *Nature*, *578*, 413–418.
- Novak, C. M., & Nunez, A. A. (1998). Daily rhythms in fos activity in the rat ventrolateral preoptic area and midline thalamic nuclei. *Am. J. Physiol.*, *275*, R1620–6.
- Novak, C. M., & Nunez, A. A. (2000). A sparse projection from the suprachiasmatic nucleus to the sleep active ventrolateral preoptic area in the rat. *Neuroreport*, *11*, 93–96.
- Nutt, D., Erritzoe, D., & Carhart-Harris, R. L. (2020). Psychedelic psychiatry’s brave new world. *Cell*, *181*, 24–28.
- O’Donnell, K. C., Roberts, D. E., Ching, T. H. W., Glick, G., Goldway, N., Gukasyan, N., Hokansen, J., Kelmendi, B., Ross, S., Yaden, M. E., & Pittenger, C. (2023). What is in a name? the many meanings of “psychedelic”. *Psychedelic Medicine*, *1*, 187–189.
- O’Hanlon, J. F., Robbe, H. W. J., Vermeeren, A., van Leeuwen, C., & Danjou, P. E. (1998). Venlafaxine’s effects on healthy volunteers’ driving, psychomotor, and vigilance performance during 15-day fixed and incremental dosing regimens. *J. Clin. Psychopharmacol.*, *18*, 212–221.
- O’Keefe, J. (1993). Hippocampus, theta, and spatial memory. *Curr. Opin. Neurobiol.*, *3*, 917–924.
- O’Keefe, J., & Recce, M. L. (1993). Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus*, *3*, 317–330.
- Oken, B. S., Salinsky, M. C., & Elsas, S. M. (2006). Vigilance, alertness, or sustained attention: Physiological basis and measurement. *Clin. Neurophysiol.*, *117*, 1885–1901.
- O’Neill, B. V., Croft, R. J., Leung, S., Guille, V., Galloway, M., Phan, K. L., & Nathan, P. J. (2006). Dopamine receptor stimulation does not modulate the

- loudness dependence of the auditory evoked potential in humans. *Psychopharmacology (Berl.)*, 188, 92–99.
- Orłowski, P., Hobot, J., Ruban, A., Szczypiński, J., & Bola, M. (2023). The relation between naturalistic use of psychedelics and perception of emotional stimuli: An event-related potential study comparing non-users and experienced users of classic psychedelics. *J. Psychopharmacol.*, 2698811231216322.
- Osmond, H. (1957). A review of the clinical effects of psychotomimetic agents. *Ann. N. Y. Acad. Sci.*, 66, 418–434.
- Osório, F. d. L., Sanches, R. F., Macedo, L. R., Santos, R. G. d., Maia-de-Oliveira, J. P., Wichert-Ana, L., Araujo, D. B. d., Riba, J., Crippa, J. A., & Hallak, J. E. (2015). Antidepressant effects of a single dose of ayahuasca in patients with recurrent depression: A preliminary report. *Rev. Bras. Psiquiatr.*, 37, 13–20.
- Osorio-Forero, A., Cardis, R., Vantomme, G., Guillaume-Gentil, A., Katsioudi, G., Devenoges, C., Fernandez, L. M. J., & Lüthi, A. (2021). Noradrenergic circuit control of non-REM sleep substates. *Curr. Biol.*, 31, 5009–5023.e7.
- Attention, n. meanings, etymology and more* [Oxford english dictionary]. (2024).
- Padilla, R., & Domina, A. (2016). Effectiveness of sensory stimulation to improve arousal and alertness of people in a coma or persistent vegetative state after traumatic brain injury: A systematic review. *Am. J. Occup. Ther.*, 70, 7003180030p1–8.
- Palagini, L., Baglioni, C., Ciapparelli, A., Gemignani, A., & Riemann, D. (2013). REM sleep dysregulation in depression: State of the art. *Sleep Med. Rev.*, 17, 377–390.
- Parent, M., & Descarries, L. (2020). Ultrastructure of the serotonin innervation in mammalian central nervous system. In *Handbook of behavioral neuroscience* (pp. 49–90, Vol. 31). Elsevier.
- Pashler, H., Johnston, J. C., & Ruthruff, E. (2001). Attention and performance. *Annu. Rev. Psychol.*, 52, 629–651.
- Pashler, H. (1990). Do response modality effects support multiprocessor models of divided attention? *Journal of experimental psychology. Human perception and performance*, 16, 826–842.
- Pazos, A., Cortés, R., & Palacios, J. M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. serotonin-2 receptors. *Brain Res.*, 346, 231–249.
- Pennes, H. H. (1954). Clinical reactions of schizophrenics to sodium amytal, pervitin hydrochloride, mescaline sulfate, and d-lysergic acid diethylamide (LSD25). *J. Nerv. Ment. Dis.*, 119, 95–112.
- Pessah, M. A., & Roffwarg, H. P. (1972). Spontaneous middle ear muscle activity in man: A rapid eye movement sleep phenomenon. *Science*, 178, 773–776.
- Pflug, B., & Tolle, R. (1971). Therapy for endogenous depression by means of sleep deprivation. *Der Nervenarzt*, 42, 117–124.
- Pickles, J. O. (2015). Auditory pathways: Anatomy and physiology. *Handb. Clin. Neurol.*, 129, 3–25.
- Piéron, H. (1913). *Le problème physiologique du sommeil*. Masson et Cie.
- Pinheiro, A. P., Rezaii, N., Rauber, A., Nestor, P. G., Spencer, K. M., & Niznikiewicz, M. (2017). Emotional self-other voice processing in schizophrenia and its relationship with hallucinations: ERP evidence. *Psychophysiology*, 54, 1252–1265.
- Pollan, M. (2019, May 30). *How to change your mind: The new science of psychedelics*. Penguin Books.



- Porkka-Heiskanen, T., & Kalinchuk, A. V. (2011). Adenosine, energy metabolism and sleep homeostasis. *Sleep Med. Rev.*, *15*, 123–135.
- Portas, C. M., Thakkar, M., Rainnie, D., & McCarley, R. W. (1996). Microdialysis perfusion of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in the dorsal raphe nucleus decreases serotonin release and increases rapid eye movement sleep in the freely moving cat. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *16*.
- Poulet, J. F. A., & Petersen, C. C. H. (2008). Internal brain state regulates membrane potential synchrony in barrel cortex of behaving mice. *Nature*, *454*, 881–885.
- Preller, K. H., & Vollenweider, F. X. (2018). Phenomenology, structure, and dynamic of psychedelic states. *Curr. Top. Behav. Neurosci.*, *36*, 221–256.
- Privitera, M., Ferrari, K. D., von Ziegler, L. M., Sturman, O., Duss, S. N., Floriou-Servou, A., Germain, P.-L., Vermeiren, Y., Wyss, M. T., De Deyn, P. P., Weber, B., & Bohacek, J. (2020). A complete pupillometry toolbox for real-time monitoring of locus coeruleus activity in rodents. *Nat. Protoc.*, *15*, 2301–2320.
- Purves, D. (2012, March 30). *Neuroscience* (D. Purves, G. J. Augustine, W. C. Hall, A.-S. LaMantia, & L. E. White, Eds.; 5th ed.). Sinauer Associates.
- Puumala, T., & Sirviö, J. (1998). Changes in activities of dopamine and serotonin systems in the frontal cortex underlie poor choice accuracy and impulsivity of rats in an attention task. *Neuroscience*, *83*, 489–499.
- Qiu, M.-H., Chen, M. C., & Lu, J. (2015). Cortical neuronal activity does not regulate sleep homeostasis. *Neuroscience*, *297*, 211–218.
- Ralph, M. R., Foster, R. G., Davis, F. C., & Menaker, M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. *Science*, *247*, 975–978.
- Rattenborg, N. C., Amlaner, C. J., & Lima, S. L. (2000). Behavioral, neurophysiological and evolutionary perspectives on unihemispheric sleep. *Neurosci. Biobehav. Rev.*, *24*, 817–842.
- Rattenborg, N. C. (2017). Sleeping on the wing. *Interface Focus*, *7*, 20160082.
- Rattenborg, N. C., & Ungurean, G. (2023). The evolution and diversification of sleep. *Trends Ecol. Evol.*, *38*, 156–170.
- Rechtschaffen, A., Bergmann, B. M., Everson, C. A., Kushida, C. A., & Gilliland, M. A. (2002). Sleep deprivation in the rat: X. integration and discussion of the findings. 1989. *Sleep*, *25*, 68–87.
- Reimer, J., McGinley, M. J., Liu, Y., Rodenkirch, C., Wang, Q., McCormick, D. A., & Tolia, A. S. (2016). Pupil fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. *Nat. Commun.*, *7*, 13289.
- Renouard, L., Billwiller, F., Ogawa, K., Clément, O., Camargo, N., Abdelkarim, M., Gay, N., Scoté-Blachon, C., Touré, R., Libourel, P.-A., Ravassard, P., Salvert, D., Peyron, C., Claustat, B., Léger, L., Salin, P., Malleret, G., Fort, P., & Luppi, P.-H. (2015). The supramammillary nucleus and the claustrum activate the cortex during REM sleep. *Science Advances*, *1*.
- Reppert, S. M., & Weaver, D. R. (2002). Coordination of circadian timing in mammals. *Nature*, *418*, 935–941.
- Rheinberger, M. B., & Jasper, H. H. (1937). Electrical activity of the cerebral cortex in the unanesthetized cat. *American Journal of Physiology-Legacy Content*, *119*, 186–196.
- Riad, M., Garcia, S., Watkins, K. C., Jodoin, N., Doucet, É., Langlois, X., El Mestikawy, S., Hamon, M., & Descarries, L. (2000). Somatodendritic

- localization of 5-HT1A and preterminal axonal localization of 5-HT1B serotonin receptors in adult rat brain. *J. Comp. Neurol.*, *417*, 181.
- Rial, R. V., Nicolau, M. C., Gamundí, A., Akaâr, M., Aparicio, S., Garau, C., Tejada, S., Roca, C., Gené, L., Moranta, D., & Esteban, S. (2007). The trivial function of sleep. *Sleep Med. Rev.*, *11*, 311–325.
- Riemann, D., Baglioni, C., Bassetti, C., Bjorvatn, B., Dolenc Groselj, L., Ellis, J. G., Espie, C. A., Garcia-Borreguero, D., Gjerstad, M., Gonçalves, M., Hertenstein, E., Jansson-Fröjmark, M., Jennum, P. J., Leger, D., Nissen, C., Parrino, L., Paunio, T., Pevernagie, D., Verbraecken, J., ... Spiegelhalter, K. (2017). European guideline for the diagnosis and treatment of insomnia. *J. Sleep Res.*, *26*, 675–700.
- Riga, M. S., Bortolozzi, A., Campa, L., Artigas, F., & Celada, P. (2016). The serotonergic hallucinogen 5-methoxy-N,N-dimethyltryptamine disrupts cortical activity in a regionally-selective manner via 5-HT1A and 5-HT2A receptors. *Neuropharmacology*, *101*, 370–378.
- Riga, M. S., Lladó-Pelfort, L., Artigas, F., & Celada, P. (2018). The serotonin hallucinogen 5-MeO-DMT alters cortico-thalamic activity in freely moving mice: Regionally-selective involvement of 5-HT 1A and 5-HT 2A receptors. *Neuropharmacology*, *142*, 219–230.
- Riga, M. S., Soria, G., Tudela, R., Artigas, F., & Celada, P. (2014). The natural hallucinogen 5-MeO-DMT, component of ayahuasca, disrupts cortical function in rats: Reversal by antipsychotic drugs. *Int. J. Neuropsychopharmacol.*, *17*, 1269–1282.
- Rimbaud, L., Passouant, P., & Cadilhac, J. (1955). Participation de l'hippocampe a la régulation des états de veille et de sommeil. [Participation of the hippocampus in regulation of sleep and wakeful states]. *Rev. Neurol. (Paris)*, *93*, 303–308.
- Robbins, T. W. (1997). Arousal systems and attentional processes. *Biol. Psychol.*, *45*, 57–71.
- Robbins, T. W. (2002). The 5-choice serial reaction time task: Behavioural pharmacology and functional neurochemistry. *Psychopharmacology*, *163*, 362–380.
- Rolls, A., Colas, D., Adamantidis, A., Carter, M., Lanre-Amos, T., Heller, H. C., & de Lecea, L. (2011). Optogenetic disruption of sleep continuity impairs memory consolidation. *Proc. Natl. Acad. Sci. U. S. A.*, *108*, 13305–13310.
- Roš, H., Sachdev, R., Yu, Y., Šestan, N., & McCormick, D. (2009). Neocortical networks entrain neuronal circuits in cerebellar cortex. *J. Neurosci.*, *29*, 10309–10320.
- Ross, S., Bossis, A., Guss, J., Agin-Liebes, G., Malone, T., Cohen, B., Mennenga, S. E., Belser, A., Kalliontzi, K., Babb, J., Su, Z., Corby, P., & Schmidt, B. L. (2016). Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: A randomized controlled trial. *J. Psychopharmacol.*, *30*, 1165–1180.
- Roßler, D. C., Kim, K., De Agro, M., Jordan, A., Giovanni Galizia, C., & Shamble, P. S. (2022). Regularly occurring bouts of retinal movements suggest an REM sleep-like state in jumping spiders. *Proc. Natl. Acad. Sci. U. S. A.*, *119*, e2204754119.
- Rucker, J. J. H., Iliff, J., & Nutt, D. J. (2018, November 1). Psychiatry & the psychedelic drugs. past, present & future.
- Rules, terminology and technical specifications. (2017). In R. B. Berry, R. Brooks, C. E. Gamaldo, S. M. Harding, C. Marcus, & B. V. Vaughn (Eds.), *The aasm*

- manual for the scoring of sleep and associated events* (p. 176). The American Academy of Sleep Medicine.
- Šabanović, M., Lazari, A., Blanco-Pozo, M., Tisca, C., Tachrount, M., Martins-Bach, A. B., Lerch, J. P., Walton, M. E., & Bannerman, D. M. (2024). Lasting dynamic effects of the psychedelic 2,5-dimethoxy-4-iodoamphetamine ((±)-DOI) on cognitive flexibility. *Mol. Psychiatry*, 1–14.
- Sacks, O. (2012, November 8). *Hallucinations*. Picador.
- Sakai, K. (2011). Sleep-waking discharge profiles of dorsal raphe nucleus neurons in mice. *Neuroscience*, 197, 200–224.
- Sallanon, M., Janin, M., Buda, C., & Jouvet, M. (1983). Serotonergic mechanisms and sleep rebound. *Brain Res.*, 268, 95–104.
- Sanches, R. F., de Lima Osório, F., Dos Santos, R. G., Macedo, L. R. H., Maia-de-Oliveira, J. P., Wichert-Ana, L., de Araujo, D. B., Riba, J., Crippa, J. A. S., & Hallak, J. E. C. (2016). Antidepressant effects of a single dose of ayahuasca in patients with recurrent depression: A SPECT study: A SPECT study. *J. Clin. Psychopharmacol.*, 36, 77–81.
- Sanchez-Vives, M. V., & McCormick, D. A. (2000). Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat. Neurosci.*, 3, 1027–1034.
- Sandison, R. A., Spencer, A. M., & Whitelaw, J. D. (1954). The therapeutic value of lysergic acid diethylamide in mental illness. *J. Ment. Sci.*, 100, 491–507.
- Sandison, R. A., & Whitelaw, J. D. (1957). Further studies in the therapeutic value of lysergic acid diethylamide in mental illness. *J. Ment. Sci.*, 103, 332–343.
- Sapin, E., Lapray, D., Bérod, A., Goutagny, R., Léger, L., Ravassard, P., Clément, O., Hanriot, L., Fort, P., & Luppi, P.-H. (2009). Localization of the brainstem GABAergic neurons controlling paradoxical (REM) sleep. *PLoS One*, 4, e4272.
- Sarasso, S., D'Ambrosio, S., Fecchio, M., Casarotto, S., Viganò, A., Landi, C., Mattavelli, G., Gosseries, O., Quarenghi, M., Laureys, S., Devalle, G., Rosanova, M., & Massimini, M. (2020). Local sleep-like cortical reactivity in the awake brain after focal injury. *Brain*, 143, 3672–3684.
- Satoh, S., Matsumura, H., Suzuki, F., & Hayaishi, O. (1996). Promotion of sleep mediated by the A2a-adenosine receptor and possible involvement of this receptor in the sleep induced by prostaglandin D2 in rats. *Proc. Natl. Acad. Sci. U. S. A.*, 93, 5980–5984.
- Sauer, S., Kinkelin, M., Herrmann, E., & Kaiser, W. (2003). The dynamics of sleep-like behaviour in honey bees. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.*, 189, 599–607.
- Scammell, T. E., Gerashchenko, D. Y., Mochizuki, T., McCarthy, M. T., Estabrooke, I. V., Sears, C. A., Saper, C. B., Urade, Y., & Hayaishi, O. (2001). An adenosine A2a agonist increases sleep and induces fos in ventrolateral preoptic neurons. *Neuroscience*, 107, 653–663.
- Semo, M., Peirson, S., Lupi, D., Lucas, R. J., Jeffery, G., & Foster, R. G. (2003). Melanopsin retinal ganglion cells and the maintenance of circadian and pupillary responses to light in aged rodless/coneless (rd/rd cl) mice: Melanopsin ganglion cells and light responses. *Eur. J. Neurosci.*, 17, 1793–1801.
- Shao, L.-X., Liao, C., Gregg, I., Davoudian, P. A., Savalia, N. K., Delagarza, K., & Kwan, A. C. (2021). Psilocybin induces rapid and persistent growth of dendritic spines in frontal cortex in vivo. *Neuron*, 109, 2535–2544.e4.



- Steriade, M. M., McCormick, D. A., & Sejnowski, T. J. (1993a). Thalamocortical oscillations in the sleeping and aroused brain. *Science*, *262*, 679–685.
- Steriade, M. M., Nuñez, A., & Amzica, F. (1993b). A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: Depolarizing and hyperpolarizing components. *J. Neurosci.*, *13*, 3252–3265.
- Strassman, R. J., & Qualls, C. R. (1994). Dose-response study of N,N-dimethyltryptamine in humans. I. neuroendocrine, autonomic, and cardiovascular effects. *Arch. Gen. Psychiatry*, *51*, 85–97.
- Sun-Edelstein, C., Tepper, S. J., & Shapiro, R. E. (2008). Drug-induced serotonin syndrome: A review. *Expert Opin. Drug Saf.*, *7*, 587–596.
- Thomas, C. W., Blanco-Duque, C., Bréant, B. J. B., Goodwin, G. M., Sharp, T., Bannerman, D. M., & Vyazovskiy, V. V. (2022). Psilocin acutely alters sleep-wake architecture and cortical brain activity in laboratory mice. *Transl. Psychiatry*, *12*, 77.
- Thompson, C. (2002). Onset of action of antidepressants: Results of different analyses. *Hum. Psychopharmacol.*, *17 Suppl 1*, S27–32.
- Timofeev, I., Grenier, F., Bazhenov, M., Sejnowski, T. J., & Steriade, M. M. (2000). Origin of slow cortical oscillations in deafferented cortical slabs. *Cereb. Cortex*, *10*, 1185–1199.
- Tobler, I., & Borbély, A. A. (1982). Sleep regulation after reduction of brain serotonin: Effect of p-chlorophenylalanine combined with sleep deprivation in the rat. *Sleep*, *5*, 145–153.
- Tobler, I., & Borbély, A. A. (1985). Effect of rest deprivation on motor activity of fish. *J. Comp. Physiol. A*, *157*, 817–822.
- Tobler, I., & Borbély, A. A. (1986). Sleep EEG in the rat as a function of prior waking. *Electroencephalogr. Clin. Neurophysiol.*, *64*, 74–76.
- Tobler, I., & Neuner-Jehle, M. (1992). 24-h variation of vigilance in the cockroach *blaberus giganteus*. *J. Sleep Res.*, *1*, 231–239.
- Tononi, G., & Cirelli, C. (2003). Sleep and synaptic homeostasis: A hypothesis. *Brain Res. Bull.*, *62*, 143–150.
- Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep Med. Rev.*, *10*, 49–62.
- Trulsson, M. E., & Jacobs, B. L. (1979). Raphe unit activity in freely moving cats: Correlation with level of behavioral arousal. *Brain Res.*, *163*, 135–150.
- UN. Economic and Social Council (42nd sess.) (1967). *1197 (XLII). LSD and similar substances* (research rep.). United Nations. New-York.
- UN. Economic and Social Council (44th sess.) (1968). *1294 (XLIV). urgent control measures for LSD and similar hallucinogenic substances* (research rep.). United Nations. New-York.
- Ungurean, G., Martinez-Gonzalez, D., Massot, B., Libourel, P.-A., & Rattenborg, N. C. (2021). Pupillary behavior during wakefulness, non-REM sleep, and REM sleep in birds is opposite that of mammals. *Curr. Biol.*
- Ungurean, G., & Rattenborg, N. C. (2024). A mammal and bird's-eye-view of the pupil during sleep and wakefulness. *Eur. J. Neurosci.*, *59*, 584–594.
- Urade, Y., Eguchi, N., Qu, W.-M., Sakata, M., Huang, Z.-L., Chen, J.-F., Schwarzschild, M. A., Fink, J. S., & Hayaishi, O. (2003). Sleep regulation in adenosine A2A receptor-deficient mice. *Neurology*, *61*, S94–6.

- Vaidya, V. A., Marek, G. J., Aghajanian, G. K., & Duman, R. S. (1997). 5-HT(2A) receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. *Journal of Neuroscience*, *17*, 2785–2795.
- Valatx, J. L., & Bugat, R. (1974). Facteurs génétiques dans le déterminisme du cycle veille-sommeil chez la souris. [Facteurs génétiques dans le déterminisme du cycle veille-sommeil chez la souris]. *Brain Res.*, *69*, 315–330.
- van Enkhuizen, J., Acheson, D., Risbrough, V., Drummond, S., Geyer, M. A., & Young, J. W. (2014). Sleep deprivation impairs performance in the 5-choice continuous performance test: Similarities between humans and mice. *Behav. Brain Res.*, *261*, 40–48.
- van Kronenberg, P., Milinski, L., Kruschke, Z., & de Hoz, L. (2022). Sound disrupts sleep-associated brain oscillations in rodents in a meaning-dependent manner. *Sci. Rep.*, *12*, 6051.
- van Lutterveld, R., Sommer, I. E. C., & Ford, J. M. (2011). The neurophysiology of auditory hallucinations - a historical and contemporary review. *Front. Psychiatry*, *2*, 28.
- Vanderwolf, C. H., & Robinson, T. E. (1981). Reticulo-cortical activity and behavior: A critique of the arousal theory and a new synthesis. *Behav. Brain Sci.*, *4*, 459–514.
- Vejmola, Č., Tylš, F., Piorecká, V., Koudelka, V., Kadeřábek, L., Novák, T., & Páleníček, T. (2021). Psilocin, LSD, mescaline, and DOB all induce broadband desynchronization of EEG and disconnection in rats with robust translational validity. *Transl. Psychiatry*, *11*.
- Verge, D., Daval, G., Patey, A., Gozlan, H., el Mestikawy, S., & Hamon, M. (1985). Presynaptic 5-HT autoreceptors on serotonergic cell bodies and/or dendrites but not terminals are of the 5-HT<sub>1A</sub> subtype. *Eur. J. Pharmacol.*, *113*, 463–464.
- Vivaldi, E. A., Ocampo, A., Wyneken, U., Roncagliolo, M., & Zapata, A. M. (1994). Short-term homeostasis of active sleep and the architecture of sleep in the rat. *J. Neurophysiol.*, *72*, 1745–1755.
- Vollenweider, F. X., Leenders, K. L., Scharfetter, C., Maguire, P., Stadelmann, O., & Angst, J. (1997). Positron emission tomography and fluorodeoxyglucose studies of metabolic hyperfrontality and psychopathology in the psilocybin model of psychosis. *Neuropsychopharmacology*, *16*, 357–372.
- Vollenweider, F. X., Vollenweider-Scherpenhuyzen, M. F., Bähler, A., Vogel, H., & Hell, D. (1998). Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *Neuroreport*, *9*, 3897–3902.
- Vollenweider, F. X., & Preller, K. H. (2020). Psychedelic drugs: Neurobiology and potential for treatment of psychiatric disorders. *Nat. Rev. Neurosci.*, *21*, 611–624.
- von Economo, C. (1929). Cruchet's "encéphalomyélite subaiguë diffuse" and epidemic encephalitis lethargica. *Lancet*, *214*, 121–122.
- von Economo, C. (1930). Some new methods of studying the brains of exceptional persons (encephalometry and brain casts). *J. Nerv. Ment. Dis.*, *71*, 300–302.
- Vyazovskiy, V. V., Borbély, A. A., & Tobler, I. (2000). Unilateral vibrissae stimulation during waking induces interhemispheric EEG asymmetry during subsequent sleep in the rat. *J. Sleep Res.*, *9*, 367–371.
- Vyazovskiy, V. V., Cirelli, C., Pfister-Genskow, M., Faraguna, U., & Tononi, G. (2008). Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nat. Neurosci.*, *11*, 200–208.

- Vyazovskiy, V. V., Cirelli, C., & Tononi, G. (2011a). Electrophysiological correlates of sleep homeostasis in freely behaving rats. *Prog. Brain Res.*, 193, 17–38.
- Vyazovskiy, V. V., Faraguna, U., Cirelli, C., & Tononi, G. (2009a). Triggering slow waves during NREM sleep in the rat by intracortical electrical stimulation: Effects of sleep/wake history and background activity. *J. Neurophysiol.*, 101, 1921–1931.
- Vyazovskiy, V. V., & Harris, K. D. (2013). Sleep and the single neuron: The role of global slow oscillations in individual cell rest. *Nat. Rev. Neurosci.*, 14, 443–451.
- Vyazovskiy, V. V., Olcese, U., Hanlon, E. C., Nir, Y., Cirelli, C., & Tononi, G. (2011b). Local sleep in awake rats. *Nature*, 472, 443–447.
- Vyazovskiy, V. V., Olcese, U., Lazimy, Y. M., Faraguna, U., Esser, S. K., Williams, J. C., Cirelli, C., & Tononi, G. (2009b). Cortical firing and sleep homeostasis. *Neuron*, 63, 865–878.
- Vyazovskiy, V. V., & Tobler, I. (2012). The temporal structure of behaviour and sleep homeostasis. *PLoS One*, 7.
- Vyazovskiy, V. V., Walton, M. E., Peirson, S. N., & Bannerman, D. M. (2017). Sleep homeostasis, habits and habituation. *Curr. Opin. Neurobiol.*, 44, 202–211.
- Walker, M. P., Brakefield, T., Morgan, A., Hobson, J. A., & Stickgold, R. (2002). Practice with sleep makes perfect: Sleep-dependent motor skill learning. *Neuron*, 35, 205–211.
- Walker, M. P., & van der Helm, E. (2009). Overnight therapy? the role of sleep in emotional brain processing. *Psychol. Bull.*, 135, 731–748.
- Walter, W. G. (1936). The location of cerebral tumours by electro-encephalography. *Lancet*, 228, 305–308.
- Wehr, T. A., Wirz-Justice, A., Goodwin, F. K., Duncan, W., & Gillin, J. C. (1979). Phase advance of the circadian sleep-wake cycle as an antidepressant. *Science*, 206, 710–713.
- Weitzman, E. D., & Kremen, H. (1965). Auditory evoked responses during different stages of sleep in man. *Electroencephalogr. Clin. Neurophysiol.*, 18, 65–70.
- Welsh, D. K., Logothetis, D. E., Meister, M., & Reppert, S. M. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron*, 14, 697–706.
- Whitaker, L. H. (1964a). Lysergic acid diethylamide in psychotherapy. I: Clinical aspects. *Med. J. Aust.*, 1, 5–8.
- Whitaker, L. H. (1964b). Lysergic acid diethylamide in psychotherapy. *Med. J. Aust.*, 1, 36–41.
- Wichniak, A., Wierzbicka, A., Wałęcka, M., & Jernajczyk, W. (2017, September 1). Effects of antidepressants on sleep.
- Willins, D. L., & Meltzer, H. Y. (1997). Direct injection of 5-HT<sub>2A</sub> receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. *J. Pharmacol. Exp. Ther.*, 282, 699.
- Wilson, C. J., & Groves, P. M. (1981). Spontaneous firing patterns of identified spiny neurons in the rat neostriatum. *Brain Res.*, 220, 67–80.
- Wingen, M., Kuypers, K. P. C., & Ramaekers, J. G. (2007). The role of 5-HT<sub>1a</sub> and 5-HT<sub>2a</sub> receptors in attention and motor control: A mechanistic study in healthy volunteers. *Psychopharmacology (Berl.)*, 190, 391–400.
- Wingen, M., Kuypers, K. P. C., van de Ven, V., Formisano, E., & Ramaekers, J. G. (2008). Sustained attention and serotonin: A pharmaco-fMRI study. *Hum. Psychopharmacol.*, 23, 221–230.

- Wirz-Justice, A., & Van den Hoofdakker, R. H. (1999). Sleep deprivation in depression: What do we know, where do we go? *Biol. Psychiatry*, *46*, 445–453.
- Wolansky, T., Clement, E. A., Peters, S. R., Palczak, M. A., & Dickson, C. T. (2006). Hippocampal slow oscillation: A novel EEG state and its coordination with ongoing neocortical activity. *J. Neurosci.*, *26*, 6213–6229.
- World Health Organisation. (1967, May). *Official records of the world health organization: Twentieth world health assembly* (research rep. No. No. 160 Annexe 1). United Nations. Geneva, World Health Organization.
- Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D. J., Nicholson, C., Iliff, J. J., Takano, T., Deane, R., & Nedergaard, M. (2013). Sleep drives metabolite clearance from the adult brain. *Science*, *342*, 373–377.
- Yamagata, T., Kahn, M. C., Prius-Mengual, J., Meijer, E., Sabanovi, M., Guillaumin, M. C. C., van der Vinne, V., Huang, Y. G., McKillop, L. E., Jagannath, A., Peirson, S. N., Mann, E. O., Foster, R. G., & Vyazovskiy, V. V. (2021). The hypothalamic link between arousal and sleep homeostasis in mice. *Proc. Natl. Acad. Sci. U. S. A.*, *118*.
- Yerkes, R. M., & Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *J. Comp. Neurol. Psychol.*, *18*, 459–482.
- Yetish, G., Kaplan, H., Gurven, M., Wood, B., Pontzer, H., Manger, P. R., Wilson, C., McGregor, R., & Siegel, J. M. (2015). Natural sleep and its seasonal variations in three pre-industrial societies. *Curr. Biol.*, *25*, 2862–2868.
- Yüzgeç, Ö., Prsa, M., Zimmermann, R., & Huber, D. (2018). Pupil size coupling to cortical states protects the stability of deep sleep via parasympathetic modulation. *Curr. Biol.*, *28*, 392–400.e3.
- Zanos, P., Moaddel, R., Morris, P. J., Riggs, L. M., Highland, J. N., Georgiou, P., Pereira, E. F. R., Albuquerque, E. X., Thomas, C. J., Zarate, C. A., Jr, & Gould, T. D. (2018). Ketamine and ketamine metabolite pharmacology: Insights into therapeutic mechanisms. *Pharmacol. Rev.*, *70*, 621–660.
- Zarate, C. A., Jr, Brutsche, N. E., Ibrahim, L., Franco-Chaves, J., Diazgranados, N., Cravchik, A., Selter, J., Marquardt, C. A., Liberty, V., & Luckenbaugh, D. A. (2012). Replication of ketamine's antidepressant efficacy in bipolar depression: A randomized controlled add-on trial. *Biol. Psychiatry*, *71*, 939–946.
- Zhan, Y. (2015). Theta frequency prefrontal-hippocampal driving relationship during free exploration in mice. *Neuroscience*, *300*, 554–565.
- Zhdanova, I. V., Wang, S. Y., Leclair, O. U., & Danilova, N. P. (2001). Melatonin promotes sleep-like state in zebrafish. *Brain Res.*, *903*, 263–268.