Investigating the role of inhibition in human motor learning



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ABSTRACT

Motor learning is extremely important in everyday life: from learning how to reach and grab objects to learning complex movements, like driving a car or playing the piano, most daily activities and hobbies rely on previously acquired motor skills. Increasing evidence indicates that inhibition mediated by GABA, the most common inhibitory neurotransmitter, plays a key role in learning new motor skills. Modulating GABAergic inhibition may therefore influence motor learning.

In this thesis, I investigated the role of inhibition in human motor learning by conducting two within-subject, randomised, placebo-controlled pharmacological studies, targeting distinct GABA-receptors, in young, healthy volunteers. In each study, I used a GABA-receptor agonist, hypothesising that increasing GABAergic inhibition would lead to impairments in motor learning.

Firstly, I used *baclofen*, a $GABA_B$-receptor agonist, in a pharmaco-neuroimaging study. I evaluated the participants' motor learning behaviour and their patterns of brain activity during task performance and at rest. Using a novel technique, Magnetic Resonance Spectroscopic Imaging (MRSI), I measured [GABA] in motor areas before and after task performance. I found that baclofen significantly impaired performance on a motor sequence learning task by increasing response times compared to placebo. Baclofen also changed the pattern of learning-related [GABA] dynamics during learning. Baclofen was associated with the recruitment of additional brain areas during task performance, likely as a compensatory mechanism, and with decreased functional connectivity in resting-state networks.

In the second study, I used *zolpidem*, a $GABA_A$-receptor positive allosteric modulator. I measured participants' motor learning behaviour and their cortical excitability using transcranial magnetic stimulation (TMS). Zolpidem significantly slowed down response times and impaired the retention of visuomotor adaptation skills. I found no significant effects of zolpidem on TMS metrics.

Taken together, these results enhance our understanding of the neurophysiological mechanisms underlying human motor learning. They are also clinically relevant: both baclofen and zolpidem are commonly administered medications and they might be detrimental to the motor rehabilitation of clinical populations.

This thesis is approximately 42,000 words.

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RELATED PUBLICATIONS

Extracts from Chapters 1 and 2 have been published as an invited review paper in a peer-reviewed journal:

***Grigoras IF**, Stagg CJ. Recent advances in the role of excitation-inhibition balance in motor recovery post-stroke. Fac Rev. 2021;10:58. Published 2021 Jun 23. doi:10.12703/r/10-58*

Extracts from Chapter 1 will be published as an invited review paper in a peer-reviewed journal:

***Grigoras IF**, Stagg CJ. The role of the primary motor cortex in motor control and motor learning. Journal of Physiology (in preparation)*

Extracts from Chapters 5 and 7 will soon be submitted for peer-review as a registered report:

***Grigoras IF,** Tunbridge EM, Stagg CJ. The effect of genetic polymorphisms in GAD1 and GABRA5 genes on functional connectivity. Cortex (in preparation)*

Subsets of the data presented in Chapters 3, 4 and 5 have been submitted as MSc theses by MSc students Elias Geist, Sebastian Green and Bel Richey.

A subset of the data in Chapter 6 will be submitted as an FHS report by undergraduate student Ben Luckraft.

PRIZES AND PRESENTATIONS

Part of my research presented in Chapters 3, 4 and 5 has been presented as oral presentations at the following meetings:

2022 **Nuffield Department of Clinical Neurosciences (NDCN) Annual Postgraduate Competition** – *Winner* for a 3-minute thesis presentation "The role of inhibition in human motor learning"

2021 **British Association for Psychophamacology (BAP) Summer Meeting** – selected oral presentation "The effect of baclofen on motor learning and brain chemistry"

2021 **Medical International Conference for Students (MEDICS)** – *First prize* for oral presentation "The effect of baclofen on motor learning and brain chemistry"

I have also presented these results as posters presentations at international meetings like the Organisation of Human Brain Mapping (OHBM) meeting 2020 and the Federation of European Neuroscience Societies (FENS) 2020.

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Table of Contents

ABSTRACT **2**

ACKNOWLEDGEMENTS **4**

RELATED PUBLICATIONS **6**

PRIZES AND PRESENTATIONS **7**

CHAPTER 1 - THE ROLE OF THE PRIMARY MOTOR CORTEX IN MOVEMENT AND MOTOR LEARNING **11**

1.1 THE ROLE OF M1 IN ENCODING MOVEMENT CHARACTERISTICS 11
1.1.1 Direction *11*
1.1.2 Force *14*
1.1.3 Speed *16*
1.1.4 Target muscles *18*
1.1.5 Reaction time *22*
1.1.6 Fine motor control *24*
1.2 BRAIN OSCILLATIONS IN M1 25
1.2.1 Alpha oscillations *25*
1.2.2 Beta oscillations *27*
1.2.3 Gamma oscillations *31*
1.2.4 Theta oscillations *33*
1.2.5 Technical considerations for tACS studies *34*
1.3 THE SIGNIFICANCE OF MOTOR LEARNING IN HUMAN LIFE 36
1.4 THE ROLE OF INHIBITION IN MOTOR LEARNING 37
1.5 AIMS OF THIS THESIS 39

CHAPTER 2 - METHODS **40**

2.1 BEHAVIOURAL TASKS EVALUATING MOTOR CONTROL AND MOTOR LEARNING 40
2.1.1 Action Selection Task *41*
2.1.2 Serial Reaction Time Task (SRTT) *42*
2.1.3 Visuomotor Adaptation (VMA) Task *44*
2.2 NEUROIMAGING 46
2.2.1 Functional MRI *46*
2.2.2 Magnetic Resonance Spectroscopic Imaging *48*
2.3 TRANSCRANIAL MAGNETIC STIMULATION (TMS) 52
2.3.1 Single-pulse TMS *54*
2.3.2 Paired-pulse TMS *55*
2.3.3 Limitations of TMS *56*
2.4 SUMMARY 57

CHAPTER 3 - THE EFFECT OF BACLOFEN ON MOTOR LEARNING AND MRS-DERIVED [GABA] IN MOTOR AREAS **58**

3.1 INTRODUCTION 58
3.1.1 Baclofen and motor learning *59*
3.1.2 Aim *61*
3.2 METHODS 62
3.2.1 Participants *62*
3.2.2 Experimental design *62*
3.2.3 Sensorimotor tasks *64*
3.2.4 MR Data Acquisition *70*
3.2.5 Mood questionnaires *72*
3.2.6 Working memory tests *73*
3.2.7 Statistical Analysis *73*
3.3 RESULTS 74
3.3.1 Baclofen did not significantly affect motor performance *74*
3.3.2 Baclofen significantly impaired performance on a motor sequence learning task *75*
3.3.3 Baclofen did not significantly affect visuomotor learning *77*

3.3.4 Baclofen did not significantly change pre-task [GABA] in the left primary motor cortex and premotor cortex *79*
3.3.5 Baclofen significantly affected the pattern of GABA change during motor learning *80*
3.3.6 MRSI-derived [GABA] measurements were significantly correlated with behavioural metrics *82*
3.4 DISCUSSION 85
3.4.1 The effect of baclofen on behavioural metrics *85*
3.4.2 The effect of baclofen on MRSI-derived [GABA] *88*
3.4.3 The relationship between motor function and MRSI-derived [GABA] *92*
3.5 CONCLUSION 95

CHAPTER 4 - THE EFFECT OF BACLOFEN ON PATTERNS OF BRAIN ACTIVITY DURING TASK PERFORMANCE 96

4.1 INTRODUCTION 96
4.1.1 Structural and functional connectivity *96*
4.1.2 Task-based networks *98*
4.1.3 Aim *99*
4.2 METHODS 100
4.2.1 Experimental design *100*
4.2.2 MRI Data Acquisition *100*
4.2.3 Task fMRI analysis *101*
4.2.4 Working memory tests *103*
4.2.5 Mood questionnaires *105*
4.2.6 Statistical analysis *106*
4.3 RESULTS 107
4.3.1 Mean activation maps revealed the brain areas involved in motor task performance *107*
4.3.2 Baclofen did not significantly affect brain activity during action-selection task performance *108*
4.3.3 Baclofen was associated with increased activity in frontal regions during SRTT task performance *108*
4.3.4 Baclofen affected brain activation patterns between early and late learning *110*
4.3.5 Baclofen was associated with decreased activity in the thalamus and basal ganglia during random blocks *112*
4.3.6 Baclofen affected the changes in brain activity patterns between random and sequence blocks *113*
4.3.7 Baclofen did not significantly alter performance on working memory tests *114*
4.3.8 Baclofen significantly affected contentedness, but not alertness or calm *117*
4.4 DISCUSSION 119
4.4.1 Baclofen did not significantly affect brain activation patterns during the action-selection task performance *119*
4.4.2 Baclofen was associated with significant changes in brain activity patterns during SRTT performance *120*
4.4.3 Working memory was not significantly affected by baclofen *128*
4.4.4 Baclofen did not significantly affect alertness or calmness, but it decreased participants' contentedness *129*
4.5 CONCLUSION 130

CHAPTER 5 - THE EFFECT OF BACLOFEN ON RESTING-STATE FUNCTIONAL CONNECTIVITY 131

5.1 INTRODUCTION 131
5.1.1 Resting-state networks *131*
5.1.2 The role of GABA in functional connectivity *132*
5.1.3 Aim *133*
5.2 METHODS 134
5.2.1 Experimental design *134*
5.2.2 MRI Data Acquisition *134*
5.2.3 Resting-state fMRI analysis *135*
5.2.4 Statistical analysis *137*
5.3 RESULTS 138
5.3.1 Baclofen significantly affected the strength of resting-state networks *138*

5.3.2 Baclofen decreased within-network connectivity in the sensorimotor and the visual network 140
5.3.3 Higher motor network strength was associated with higher response times with baclofen 141
5.3.4 Baclofen did not significantly affect pre-task [GABA] in the sensorimotor network 143
5.3.5 No significant correlations between motor network strength and pre-task [GABA] in motor regions 143
5.4 DISCUSSION 145
5.4.1 The effect of baclofen on resting-state functional connectivity 145
5.4.2 Sensorimotor [GABA] was not significantly affected by baclofen and did not correlate with sensorimotor network strength 150
5.5 CONCLUSION 152

CHAPTER 6 - THE EFFECT OF ZOLPIDEM ON MOTOR LEARNING AND TMS METRICS 153

6.1 INTRODUCTION 153
6.1.1 The role of GABAergic inhibition in motor learning 153
6.1.2 The role of GABAergic inhibition in tactile discrimination 154
6.1.3 Zolpidem – mechanism of action and clinical use 155
6.1.4 The effect of Zolpidem on TMS metrics 156
6.1.5 The effect of Zolpidem on motor behaviour 157
6.1.6 Aim 157
6.2 METHODS 158
6.2.1 Participants 158
6.2.2 Experimental design 158
6.2.3 Sensorimotor tasks 160
6.2.4 Transcranial Magnetic Stimulation (TMS) 167
6.2.5 Working memory tests 169
6.2.6 Mood questionnaires 171
6.2.7 Statistical analysis 172
6.3 RESULTS 173
6.3.1 Zolpidem significantly increased response times and actions-selection times 173
6.3.2 Zolpidem significantly impaired performance on the SRTT 175
6.3.3 Zolpidem significantly impaired retention of visuomotor adaptation skills 179
6.3.4 Zolpidem significantly impaired touch perception 182
6.3.5 Zolpidem did not significantly change TMS metrics of cortical excitability and GABAergic inhibition 183
6.3.6 Zolpidem significantly impaired performance on the AVLT, but not the Digit Span task ... 185
6.3.7 Zolpidem significantly decreased participants' alertness and contentedness 187
6.4 DISCUSSION 189
6.4.1 The effect of zolpidem on behavioural metrics 189
6.4.2 The effect of zolpidem on TMS metrics 196
6.4.3 Zolpidem affected the participants' mood, significantly decreasing alertness and contentedness 198
6.5 CONCLUSION 199

CHAPTER 7 - GENERAL DISCUSSION 200

7.1 SUMMARY 200
7.2 MAIN CONCLUSIONS 203
7.3 GENERAL LIMITATIONS OF PHARMACOLOGICAL STUDIES 204
7.4 FUTURE DIRECTIONS 206
7.4.1 The genetic basis for inhibition in the brain 206
7.4.2 The effect of baclofen on oscillatory activity 207
7.4.3 The effect of baclofen on the motor recovery of stroke survivors 207
7.4.4 The role of inhibition throughout stroke recovery – an animal neuroimaging study 208
7.5 CONCLUSION 210

LIST OF FIGURES 211

REFERENCES 214

CHAPTER 1 - THE ROLE OF THE PRIMARY MOTOR CORTEX IN MOVEMENT AND MOTOR LEARNING

This thesis will investigate the role of inhibition in human motor learning. In this chapter, I will first review the role of the primary motor cortex (M1) on motor control and motor execution, by describing how M1 encodes movement characteristics such as direction, force, speed and recruitment of target muscles. I will then discuss the significance of motor learning in human life and review the existing evidence on the role of inhibition in motor learning and plasticity. Finally, I will outline the aims of this thesis, as described in the following chapters.

1.1 THE ROLE OF M1 IN ENCODING MOVEMENT CHARACTERISTICS

1.1.1 Direction

Whether we reach for a cup of coffee or move the cursor on the screen using a mouse, the correct movement direction is key to a successful movement. Better understanding how the motor cortex encodes these directions can help optimise decoding algorithms and improve the performance of brain-machine interfaces (BMIs), devices that allow the movement of prosthetics from neural signals (Branco *et al.*, 2019*a*).

Early studies used invasive recording in monkeys executing a reaching task to investigate how the firing patterns of single neurons in the motor cortex related to the reach direction. Single neurons were reported to encode direction using a cosine function, the peak of the cosine representing the preferred direction (Georgopoulos *et al.*, 1982, 1988; Schwartz, 1993, 2016). However, more recent studies show that the preferred direction

of a single neuron can change with time, speed and posture (Hatsopoulos *et al.*, 2007; Churchland & Shenoy, 2007*a*; Oby *et al.*, 2013; Suminski *et al.*, 2015; Lalazar *et al.*, 2016). For example, while most neurons fit the cosine tuning function (88% with cc >0.5), the preferred direction was consistent between trials in only 13% of the analysed neurons (Wu & Hatsopoulos, 2006). These findings speak of the great complexity of cortical signals that give rise to movements: the patterns in which the preferred direction changed were generally unique to the single neuron. Moreover, when external force fields are applied, tuning curves of single neurons change so that the population activity can compensate for the perturbation (Arce *et al.*, 2010; Richardson *et al.*, 2012). Of relevance to BMI performance, reaching direction can be decoded from population activity recorded as local field potentials (Mehring *et al.*, 2003; Rickert *et al.*, 2005). The population activity has the same preferred direction as its corresponding muscle groups; within this population, there are neurons with reversed preferred direction, which likely control the activity of antagonist muscles (Heming *et al.*, 2016).

The preferred direction of single neurons does not only change between trials but within trials too (Suminski *et al.*, 2015; Suway *et al.*, 2018). (Suway *et al.*, 2018) found that preferred direction changes between 3 well-defined stages of each trial: the first stage corresponding to reaction time, the second to initial transport phase and the third to target acquisition. Within each stage, neurons generally presented a consistent preferred direction. Adding non-directional parameters to the model (position, speed, velocity, acceleration, additional kinematics) did not account for the change of preferred direction during reaching trials. Therefore, discrete processes take place consecutively during a single reach, the transition from one to another being associated with a change in

directional tuning, consistent with the findings of previous studies (Hatsopoulos *et al.*, 2007; Rouse & Schieber, 2016; Kadmon Harpaz *et al.*, 2019). The mechanisms underlying these transitions between movement sub-stages are still unclear; they might rely on changes in intrinsic M1 oscillatory activity or input from other motor areas (Jin *et al.*, 2014; Hall *et al.*, 2014).

Whether different movement features are encoded by discrete subpopulations of neurons is an important question for the field of motor control, with practical implications for BMIs. Since the same neurons have a shift in tuning during reach stages, the same population might encode different movement features throughout time: the same units are first tuned to location and then to the object (Rouse and Schieber 2016). Indeed, approximately 60% of all recorded units were tuned to target location, 78% to the object and 55% to both target and object (Rouse & Schieber, 2018). However, some motor neurons do not show directional tuning; it is likely they are either tuned to other movement characteristics or they might be neurons which do not have a direct connection to the spinal motoneurons. Indeed, technical limitations of *in vivo* invasive techniques include not being able to choose which neurons' activity to record. Neurons with direct projections to alpha motoneurons (corticomotor neurons, CMs) are more likely to show strong tuning than neurons that project onto CMs (Chaisanguanthum *et al.*, 2017), so the neurons which show no directional tuning might belong to the latter category.

1.1.2 Force

The degree of exerted force is essential for successful movements; it can make the difference between holding and breaking a glass. Considering that BMIs need to be able to decode the appropriate force for reaching and grasping an object, it is crucial to better understand how the motor cortex encodes force. An early study reported a linear relationship between the firing rates of single units and force (Evarts, 1968). However, later studies showed that the force-generating cortical mechanisms are far more complex (Ashe, 1997). Only a proportion of the recorded neurons showed a linear relationship between firing rate and force, both positive and negative linear relationships having been reported in the same neuronal population (Werner *et al.*, 1991; Wannier *et al.*, 1991; Maier *et al.*, 1998; Hepp-Reymond *et al.*, 1999; Intveld *et al.*, 2018). Similar results arise from the analysis of population-level dynamics: only some subsets correlated with muscle activity, indicating that part of the recorded population activity is involved in other processes (Intveld *et al.*, 2018). Maximal correlation between the population activity and EMG was observed when the neuronal signals preceded the EMG activity by 10ms, suggesting that this relationship was not determined by the sensory feedback (Intveld *et al.*, 2018).

In humans, fMRI activation of the motor cortex is used as a proxy for neuronal activity. Higher force is associated with higher fMRI activation volume in the contralateral motor cortex (Thickbroom *et al.*, 1998; Cramer *et al.*, 2002; Spraker *et al.*, 2007) and increases in task-related BOLD signal amplitude (Dai *et al.*, 2001; Spraker *et al.*, 2007; Ward *et al.*, 2008; Keisker *et al.*, 2009). While these studies generally report linear relationships between force and BOLD signal, a recent study found both linear and non-linear positive

effects of force on the BOLD signal (Alahmadi *et al.*, 2016). With stronger force, more muscle fibres need to be activated by increased neuronal recruitment in the contralateral M1; this may be the mechanism of the positive linear effects observed. The non-linear effects could be a result of the neuronal population including both positively and negatively force-tuned neurons, muscle fatigue or changes in attention during the task.

Further evidence for the important role of the motor cortex in force generation comes from motor imagery studies. Mental imagery of muscle contractions can increase strength, albeit less than actual motor practice (Ranganathan *et al.*, 2004) and diminish the muscle weakness associated with prolonged immobilisation (Clark *et al.*, 2014). Moreover, mirror neurons in M1 can also represent grip force (Atique & Francis, 2021).

Investigating how the motor cortex encodes force presents technical challenges, which make replicating results between studies difficult. Different tasks are used to study force production: usually centre-out, isometric or force field tasks in non-human primates, and timed squeezing tasks in humans. While they all involve the production of different levels of force, the centre-out task has a reaching component, which could bias the force-related results if recordings are acquired from neurons that are also tuned to direction. Alternatively, some of the sampled neurons might not be tuned to force and instead involved in different processes; the activity of the force-tuned neurons would therefore not be representative of the entire neuronal population. Some studies do not take into consideration the activity of non-tuned neurons, which can lead to sample bias. The location of the recording is also important, as the caudal M1 has been more implicated in force modulation than the rostral M1 (Sergio & Kalaska, 1998; Sergio *et al.*, 2005; Addou *et al.*, 2015). Recording ECoG data in patients, (Flint *et al.*, 2020) found that the mean

distance between the peak decoding location for movement kinematics and the peak decoding location for force was 9.9mm, which is important when choosing the optimal ECoG array to use for BMIs.

Grasp force can be decoded reasonably well using invasive recordings in both monkeys (Carmena *et al.*, 2003; Naufel *et al.*, 2019) and humans (Pistohl *et al.*, 2012; Flint *et al.*, 2014; Murphy *et al.*, 2016); higher individual variability in decoding accuracy was reported in humans. Attempted grasp force was also decoded with good accuracy (69%) from the M1 activity of a tetraplegic patient, which is an exciting step towards widely using BMIs in patients with motor impairments (Ajiboye *et al.*, 2017; Downey *et al.*, 2018). Better understanding how force is encoded in M1 could further refine the decoding algorithms and improve BMI performance.

1.1.3 Speed

Whether M1 encodes movement speed, velocity or neither has long been a topic of debate in the field. Studies in non-human primates now show that while speed-tuning does exist in some M1 neurons, it is much weaker than direction-tuning (Schwartz, 1992; Moran & Schwartz, 1999; Johnson et al., 1999; Churchland et al., 2006b; Aflalo & Graziano, 2007; Golub et al., 2014). The studies that found the strongest speed-tuning were the ones that recorded single-unit activity from both M1 and the PMd, without clustering the units in their respective areas. If the PMd is more directly involved in speed encoding than M1, that would explain why higher speed-tuning is found in studies investigating both brain areas (Johnson *et al.*, 1999; Churchland *et al.*, 2006*b*). Population activity generally encodes speed and velocity better than single-unit activity (Stark & Abeles, 2007; Bansal *et al.*, 2011; Perel *et al.*, 2013). Since single-units show more

direction-tuning, it is possible that different preferred directions in different cells cancel each other out at population level, highlighting the speed-tuning (Hammer *et al.*, 2016).

In humans, however, brain imaging studies show no speed-specific modulation of M1 activity, only changes related to movement *per se*; other brain areas do show changes in activity depending on speed, including the PMd, supplementary motor area, putamen, thalamus and cerebellum (Turner *et al.*, 2003; Wenzel *et al.*, 2014; Stark-Inbar & Dayan, 2017; Shirinbayan *et al.*, 2018). This finding could relate to the weak speed-tuning reported in M1 or to the limitations of fMRI techniques. The changes in the activity of speed-tuned neurons might not have been strong enough to be picked up by the BOLD signal. Additionally, each voxel includes a huge number of neurons, which are involved in processes other than speed encoding. Together with studies in non-human primates that found the strongest speed-specific activity in recordings from both PMd and M1 (Johnson *et al.*, 1999; Churchland *et al.*, 2006*b*), this indicates that speed of movement may be regulated by the cortico-basal ganglia-thalamocortical loop rather than M1 itself.

The only evidence of speed information being encoded in M1 activity comes from ECoG studies, where increases in high gamma power (80 – 160 Hz) are associated with increased speed/velocity (Wang *et al.*, 2013, 2017; Hammer *et al.*, 2016). However, this result might be due to the increased muscle recruitment associated with increased speed; gamma power would also be expected to increase with increased muscle recruitment. Indeed, isometric contractions are associated with higher gamma power than rest, despite no speed being produced in either condition, supporting the hypothesis that the increase of gamma power with speed might be a result of more extensive muscle recruitment when performing fast movements (Wang *et al.*, 2017).

However, it is not impossible that M1 encodes movement speed. For biomechanical reasons, movement speed will inevitably be correlated with other movement parameters, like limb posture, velocity (directional speed), and acceleration, which make speed-specific effects harder to disentangle. Moreover, the techniques used might be unsuited for the computations underlying speed production, either by not sampling the correct neuronal population or by not using the most appropriate computational approaches.

Taken together, these findings imply that M1 activity alone may not be enough to correctly decode speed using BMIs. Duration of movements performed with prosthetics is usually longer than with the native arm and difficulties have been reported in tasks requiring stopping at different target locations, but new decoding methods are improving BMI performance (Carmena *et al.*, 2003; Hochberg *et al.*, 2006; Kim *et al.*, 2008; Gilja *et al.*, 2011, 2012 p.201; Golub *et al.*, 2014).

1.1.4 Target muscles

1.1.4.1 Spatial clustering of functionally related neurons

Somatotopy is present in M1, with distinct representations for limbs, but within-limb somatotopy has long been questioned (Flanders, 2005). Somatotopic representations have been reported within the upper limb area, but there is also an overlap between these representations, likely related to inter-muscle and inter-joint coordination (Plow *et al.*, 2010; Cunningham *et al.*, 2013; Ejaz *et al.*, 2015; Massé-Alarie *et al.*, 2017; Schellekens *et al.*, 2018; Huber *et al.*, 2020). This overlap appears to be dynamic and has been linked to plasticity and better motor performance; the cortical representation of a muscle increases with practice and the post-stroke cortical reorganisation of the motor cortex is

associated with improved motor function (Nudo *et al.*, 1996*a*, 1996*b*; Tyč *et al.*, 2005; Tyč & Boyadjian, 2011).

For example, the upper-limb M1 neurons in non-human primates appear to be grouped into two spatial clusters, one lateral for hand representations and grasping, and one medial for arm representations and reaching; some neurons were found to be tuned to more than one phase of the reach and grasp movement (9/38) and they may have been recorded from the overlap region between these two clusters (Friedman *et al.*, 2020). Two spatial clusters related to hand movements have also been reported in a human neuroimaging study using magnetic resonance imaging (MRI) and magnetoencephalography (MEG): one caudal encoding kinematic information and one rostral related to muscle activity (Kolasinski *et al.*, 2020). However, not all studies in non-human primates found this spatial clustering pattern, potentially due to small numbers of recorded neurons, placement of the electrodes in the overlap zone or increased overlap of representations post-training (Saleh *et al.*, 2012; Rouse & Schieber, 2016; Hudson *et al.*, 2017).

1.1.4.2 *Muscle synergies*

To investigate the role of the motor cortex in muscle activation, research has focused on the cortical neurons with the quickest connection to muscles: cortico-motor neurons (CMs) (Lemon, 2019). CMs synapse onto alpha motoneurons in the spinal cord, which directly innervate muscle fibres, therefore they are the most likely neurons to directly encode patterns of muscle activation. CMs are mainly found in the caudal M1 (Rathelot & Strick, 2006, 2009) and, unlike alpha motoneurons which activate one specific muscle, they have a muscle field: by modulating the activity of more alpha motoneurons, they can

activate a group of muscles known as 'functional synergists' (Buys *et al.*, 1986; Lemon, 2008, 2021; Overduin *et al.*, 2012, 2015; Griffin *et al.*, 2015). Functional synergies are patterns of co-activation of different muscles engaged in the same movement; the most simple example is the agonist-antagonist co-activation, where the agonist produces the movement execution and the antagonist controls the end of the movement (Latash, 2018). EMG-derived muscle synergies are thought to have a cortical origin, as different synergies are reported in the left and right arm, despite identical tasks and biomechanics (Bizzi & Cheung, 2013; Pellegrino *et al.*, 2020).

CMs control the function of the muscle in the synergy: the same muscle can be activated by different sets of CMs depending on its function as agonist/synergist/fixator/antagonist (Griffin *et al.*, 2015). Functional tuning of CMs has been previously reported for grip types (Muir & Lemon, 1983); it may allow more flexible muscle recruitment and increase the complexity of possible movements. Whether functional tuning is an intrinsic property of CMs or a learnt feature is still unclear (Tresch & Jarc, 2009), although synergies can be gradually created throughout development (Dominici *et al.*, 2011). Synergies are mostly conserved between subjects into adulthood (Torres-Oviedo & Ting, 2007, 2010; Overduin *et al.*, 2008; Hug *et al.*, 2011; Amundsen Huffmaster *et al.*, 2018), but there are also reports of subject-specific voluntary synergies (Ajiboye *et al.*, 2017; Amundsen Huffmaster *et al.*, 2017). Simpler synergies may be consistent between adult subjects due to biomechanical constraints or similar patterns of movement (reach and grasp, walking), while more complex synergies may be subject-specific.

1.1.4.3 The neural manifold

Neurons in the motor cortex do not fire independently from each other; they are connected to both other neurons in the motor cortex and neurons from other brain regions. However, studies recording from single units do not account for this and try to find links between firing rates of individual neurons and movement parameters, without considering network activity. In most of the single neuron studies discussed above, a part of the recorded neurons was not tuned to any movement feature, indicating other cortical processes taking place. Since the role of M1 is movement production, single neurons need not represent specific movement characteristics, as long as the network produces the appropriate output signals (Churchland *et al.*, 2012).

Technical developments now allow us to record the activity of large numbers of neurons and use computational techniques to model their interactions (Ganguli & Sompolinsky, 2012; Gao & Ganguli, 2015). To investigate population dynamics, the concept of the neural manifold has been described as a low-dimensional space composed of fundamental computational units called neural modes, which contain a fraction of the total neuronal population (Santhanam *et al.*, 2009; Churchland *et al.*, 2012; Gallego *et al.*, 2017). The same neuron may be part of more neural modes, its activity being a projection of the population activity (Gallego *et al.*, 2017). The structure of the neural modes does not change depending on the executed task and both task-dependent and task-independent neural modes have been reported; the activity of some task-independent modes closely resembled the muscle activity (Gallego *et al.*, 2018). It has been proposed that the complexity of movement behaviour might be achieved by flexibly activating different combinations of neural modes (Gallego *et al.*, 2018). For practised behaviour,

the time-dependent activation of neural modes is stable over time (Gallego *et al.*, 2020). The dimensionality of the neural manifold may however depend on sensory input, as it is not low-dimensional during grasp like it is during reach or centre-out pointing (Churchland *et al.*, 2012; Pandarinath *et al.*, 2015; Suresh *et al.*, 2020). This change in dimensionality may also arise from different kinematics between grasp and reach or much higher dimensionality of M1 activity during grasp (Suresh *et al.*, 2020). Whether neural modes underlie the activity of other brain regions is still unclear.

Population activity is also important for learning new motor skills. Using a closed-loop intracortical BMI, (Sadtler *et al.*, 2014) found that motor learning was improved when neural activity followed the intrinsic neural manifold, indicating that the structure of the neural modes affects motor learning. Moreover, long-term learning is associated with the generation of new neural modes, likely through mechanisms of neural plasticity (Oby *et al.*, 2019). Future studies could further investigate whether plasticity induction does lead to the generation of new neural modes and improved task performance and how generalisable this motor improvement is between different motor tasks. It is still unclear how activation of neural modes changes in pathological conditions.

1.1.5 Reaction time

Although the premotor cortex is generally recognised as the brain area controlling motor planning and timing of movements (Churchland *et al.*, 2006*c*, 2006*a*; Kantak *et al.*, 2012; Shenoy *et al.*, 2013), M1 also has a role in movement preparation: neural activity changes in M1 before movement onset (Tanji & Evarts, 1976) and this change is related to reaction times (Riehle & Requin, 1993; Bastian *et al.*, 2003). Altering M1 excitability using non-invasive brain stimulation techniques (repetitive transcranial magnetic stimulation, rTMS)

affects movement initiation: inhibitory rTMS to M1 leads to decreased pre-movement activity in M1 (Rossi *et al.*, 2000) and slower reaction times (Jäncke *et al.*, 2004; Terao *et al.*, 2007; Di Lorenzo *et al.*, 2013).

Moreover, there is a link between inhibition in M1 and response times: higher [GABA] in M1 is associated with slower response times in healthy people (Stagg *et al.*, 2011*b*).

Differences in both individual neurons and population activity occur just before movement onset. For individual neurons, tuning changes approximately 150ms before movement (Churchland *et al.*, 2010); for population activity, its dynamics change from stable to rotational just before movement (Churchland *et al.*, 2012), indicating that a 'triggering' stimulus is likely to occur then (Sussillo *et al.*, 2015). This stimulus is likely unrelated to the type of movement to be executed (Hennequin *et al.*, 2014). Indeed, (Kaufman *et al.*, 2016) found a condition-invariant stimulus (not tuned to reach speed, distance and curvature), which explained more of M1 activity than any of the tuned components. Future studies should further investigate the origin of this stimulus; it could be intrinsic to M1, but it could also originate from a different brain area connected to M1, such as the parietal cortex, supplementary motor area, basal ganglia, or the cerebellum. The changes in pre-movement M1 activity may also contribute to the 'functional tuning' of M1: while the CM cell activates the motoneuron for the agonist muscle, it can also disynaptically inhibit the motoneuron for the corresponding antagonist muscle (Cheney *et al.*, 1985; Maier *et al.*, 1998; Takei & Seki, 2010; Griffin & Strick, 2020), a process referred to as the Hufschmidt phenomenon (Hufschmidt & Hufschmidt, 1954; Berardelli *et al.*, 1996).

1.1.6 Fine motor control

The human hand can do much more skilled movements than grasping – professional musicians can achieve extraordinary precision and speed using their hands. The hand area in the motor cortex represents a much higher proportion of the motor cortex than its size compared to the rest of the body, reflecting the complexity of motor control of the hand (Mignano & Konz, 1994; Mayer *et al.*, 2019). For recent reviews on the role of the cortex in hand movement, please see (Isa, 2019; Sobinov & Bensmaia, 2021).

The monosynaptic connection between corticomotor neurons (CMs) and the alpha-motoneuron is unique to primates (Lemon & Griffiths, 2005) and has been linked to our abilities to skillfully use our hands and manipulate tools (Heffner & Masterton, 1983; Bortoff & Strick, 1993; Iwaniuk *et al.*, 1999; Quallo *et al.*, 2012; Lemon, 2021). This link is supported by findings in rodents, where grasping and manipulation behaviour is improved in genetically engineered mice that have CMs preserved from early to adult life (Gu *et al.*, 2017). The activity of one alpha-motoneuron is influenced by multiple CMs which can activate the target muscle as an agonist/fixator/synergist/antagonist, allowing more flexibility compared to hard-wired spino-motor connections (Griffin *et al.*, 2015).

Fine motor control is closely linked to the somatotopic representation of fingers in M1; finger movements that are highly correlated during everyday activities in humans reveal similar fMRI activity patterns (Ingram *et al.*, 2008; Ejaz *et al.*, 2015). Whether different movements in the same finger (flexion/extension) have separate cortical representations is still under debate, as recent fMRI studies show conflicting results (Huber *et al.*, 2020; Arbuckle *et al.*, 2020). This is likely due to limitations in fMRI spatial and temporal

resolution, as well as differences in task paradigms such as whole-hand movements versus individual finger movements.

Fine motor control is particularly important for the neurorehabilitation of stroke survivors, who report having the most difficulties performing bimanual activities that require manual dexterity (Ekstrand *et al.*, 2016). Therefore, future motor rehabilitation programs should design new physiotherapy interventions to specifically target these deficits (Collins *et al.*, 2018; Parry *et al.*, 2019; Kantak & Luchmee, 2020).

1.2 Brain oscillations in M1

Oscillatory brain function is classified into 5 frequency bands: alpha (8 – 12 Hz), beta (13 – 30 Hz), gamma (30 – 110 Hz), delta (0.5 – 4 Hz) and theta (4 – 8 Hz), each with distinct areal representations and roles. Peak frequencies for different brain regions have been recently assembled in a brain atlas and replicated in two individual sub-samples (Capilla *et al.*, 2021). While the beta and gamma bands seem to be the most important for motor control, we will briefly discuss the role of each frequency band in movement execution and motor learning.

1.2.1 Alpha oscillations

Alpha or mu activity is characterised by oscillatory activity between 8 – 12 Hz, its frequency changing with age, processing of sensorimotor information and cognitive state (Cheyne, 2013; Mierau *et al.*, 2017). The role of alpha oscillations is thought to be connected to visual and auditory perception (Bauer *et al.*, 2012; Romei *et al.*, 2012; Cecere *et al.*, 2015; Samaha & Postle, 2015).

In humans, the role of alpha activity in motor control has been investigated using transcranial alternating current stimulation (tACS), a non-invasive brain stimulation technique that uses weak alternating current to stimulate the cortex at specific frequencies. However, increasing evidence from both EEG and MEG studies now shows that alpha-tACS has no effect on intrinsic alpha activity after the stimulation (Wach *et al.*, 2013; Sugata *et al.*, 2018; Harada *et al.*, 2020; Lafleur *et al.*, 2021). Although tACS is thought to entrain the cortical neurons of the stimulated region, an alpha-tACS study in monkeys reported that only a minority of the neurons showed entrainment, but their proportion increased with current intensity (8.9% for 0.5mA to 26.5% for 1.5mA; (Johnson *et al.*, 2020)). The fact that such a small proportion of neurons shows entrainment to alpha-tACS might be the reason why we do not see an overall population effect of alpha-tACS in humans.

The effect of alpha-tACS on motor behaviour is still being investigated. Alpha-tACS increased variability of motor performance 30 minutes after the stimulation but was not associated with significant changes in reaction times (Wach *et al.*, 2013). One early study linked alpha-tACS to improved implicit motor learning, but this finding has not been replicated (Antal *et al.*, 2008; Sugata *et al.*, 2018). This may be due to technical differences between the two studies: (Antal *et al.*, 2008) reported improved motor learning during alpha-tACS, while (Sugata *et al.*, 2018) had participants perform the motor learning task before and after stimulation. Maximal behavioural effects may be achieved during stimulation, which do not persist post-stimulation.

1.2.2 Beta oscillations

Beta activity is characterised by dynamic activity between 13 – 30 Hz and has been heavily studied in motor control and movement production (Engel & Fries, 2010). Beta activity has been reported in brain areas that control motor functions, such as the sensorimotor cortex and basal ganglia (Jensen *et al.*, 2005*b*; Roopun *et al.*, 2006; Holgado *et al.*, 2010; McCarthy *et al.*, 2011; Barone & Rossiter, 2021). However, whether it represents an intrinsic property of each brain region or whether it stems from their connection is still unclear (Sherman *et al.*, 2016; Reis *et al.*, 2019). For a detailed review of the role of beta oscillations in health and disease, please see (Barone & Rossiter, 2021).

1.2.2.1 Event-related changes in beta activity

Movement execution is associated with changes in beta power in the sensorimotor area: beta power decreases before and during movement production (event-related desynchronization, ERD) and then temporarily rebounds upon movement completion (event-related synchronization, ERS (Neuper & Pfurtscheller, 2001; van Wijk *et al.*, 2012). These movement-related changes are associated with GABAergic inhibition: administration of $GABA_A$ receptor agonist diazepam increased ERDs, but not ERSs (Hall *et al.*, 2011), while increasing GABA levels with GABA reuptake inhibitor tiagabine affected both ERDs and ERSs (Muthukumaraswamy *et al.*, 2013). ERDs and ERSs may therefore involve activation of different GABAergic receptors. Moreover, changes in neuronal firing rates and tuning properties have been reported at movement onset; these changes might make the neuronal population more heterogeneous and less synchronised, giving rise to ERDs (Churchland & Shenoy, 2007*a*; Suminski *et al.*, 2015; Best *et al.*, 2016).

Both resting-state beta power and movement-related changes in beta power show high within-subject reliability, which may encourage their use in longitudinal studies with clinical populations (Pollock *et al.*, 1991; Näpflin *et al.*, 2007; Martín-Buro *et al.*, 2016; Espenhahn *et al.*, 2017). Muscle contraction is not required for the presence of ERDs; they may also appear, to a smaller extent, when movement is observed or imagined (Miller *et al.*, 2010; Nakagawa *et al.*, 2011; Kilavik *et al.*, 2013). They could therefore be of particular interest in studies with patients who have impaired motor function.

In (Espenhahn *et al.*, 2017), the only measurement that showed low within-subject reliability was the beta peak frequency. In other frequency bands, individual peak frequency is thought to be more reliable and twin studies indicate that it is genetically determined (van Beijsterveldt & van Baal, 2002; van Pelt *et al.*, 2012). Even if the factors that determine the individual beta peak frequency are still unclear, finding it is important for optimal brain stimulation protocols. Repetitive TMS (rTMS) over the motor cortex at the individual beta peak frequency, but not at surrounding frequencies, increases beta power, cortical phase-locking and corticospinal coherence (Romei *et al.*, 2016). Future brain stimulation studies should consider personalizing the stimulation protocol with each person's peak frequency (Schoenfeld *et al.*, 2021), instead of stimulating all participants at a fixed frequency.

1.2.2.2 The role of beta activity in motor behaviour

Using non-invasive brain stimulation to increase cortical beta activity leads to detrimental changes in motor behaviour. Beta tACS is associated with slower voluntary movement and slower force generation (Pogosyan *et al.*, 2009; Joundi *et al.*, 2012); continuous theta-burst stimulation (cTBS) can increase beta power, leading to slower reaction times and

disruptions in force generation (Huang *et al.*, 2005; Nowak *et al.*, 2005; McAllister *et al.*, 2013). Therefore, beta activity is hypothesised to have an anti-kinetic role, thought to maintain the *status quo* of the sensorimotor network (Engel & Fries, 2010). However, non-significant effects of modulating beta activity on motor performance have also been reported (Schabrun *et al.*, 2008; Moisa *et al.*, 2016; Santarnecchi *et al.*, 2017; Miyaguchi *et al.*, 2018; Guerra *et al.*, 2018; Schoenfeld *et al.*, 2021), likely due to technical difference in stimulation parameters and motor tasks used. Limitations of tACS studies are discussed in more detail below.

The anti-kinetic role of beta activity is further supported by micro-stimulation experiments in non-human primates. At movement onset, the propagation of the spatio-temporal ERD is aligned with the successive activation of single units (Best *et al.*, 2016). Intracortical micro-stimulation can be used to create patterns of propagating currents in the cortex; when these induced currents oppose the physiological beta desynchronization wave, reaction times become slower (Balasubramanian *et al.*, 2020), emphasizing that ERDs are important for normal motor function. Moreover, motor learning is also associated with more pronounced ERDs (Zich *et al.*, 2018). It is still unclear how the beta desynchronization wave relates to muscle recruitment. Further studies could investigate its effect on subcortical structures and intracortical (horizontal) connectivity.

Non-human primate studies show that monkeys can be trained to modulate their endogenous beta activity in M1 during a motor task; larger decreases in beta power are associated with faster reaction times (Khanna & Carmena, 2017). Training patients to modulate their beta activity using neurofeedback might present advantages over brain stimulation techniques: it would allow patients to use individualised strategies; it would

be more comfortable and have fewer side effects; once the strategy has been learnt, patients could use it in day-to-day life to maximise long-term effects.

1.2.2.3 Beta oscillations as burst activity

Recently, our understanding of beta oscillations has been reshaped by numerous studies proposing that the temporal character of beta activity is in fact transient bursts of 100 – 200ms, rather than ongoing oscillations (Leventhal *et al.*, 2012; Feingold *et al.*, 2015; Sherman *et al.*, 2016; Jones, 2016; Shin *et al.*, 2017; Ede *et al.*, 2018; Torrecillos *et al.*, 2018; Little *et al.*, 2019; Tinkhauser *et al.*, 2020; Zich *et al.*, 2020). ERDs/ERSs are therefore more likely to represent decreases/increases in the probability of a burst occurring rather than changes in a stable rhythm (Feingold *et al.*, 2015). The trial average beta amplitude in ERD and ERS periods is correlated with the burst probability and the timing of the beta burst predicts motor behaviour better than burst rate or single-trial beta-amplitude (Little *et al.*, 2019). Consistent with the proposed anti-kinetic role of beta activity, late pre-movement beta bursts are associated with a slower reaction time (Little *et al.*, 2019). In future studies, analysis of single-trial burst dynamics may be preferable to trial averaged data, as single-trial bursts seem to be more informative towards behaviour. For a recent review on oscillatory bursting, please see (Tal *et al.*, 2020).

1.2.3 Gamma oscillations

Gamma activity is characterised by oscillatory activity between 30 – 110 Hz, generated by the activation of a network of interconnected excitatory pyramidal neurons and inhibitory interneurons, of which fast-spiking parvalbumin interneurons are of particular importance (Sohal *et al.*, 2009; Buzsáki & Wang, 2012; Nowak *et al.*, 2018). Modulating gamma activity with gamma-tACS is associated with a decrease in $GABA_A$-mediated inhibition (Nowak *et al.*, 2017), highlighting the role of GABAergic processes in gamma activity.

Movement execution is associated with changes in gamma power in the sensorimotor area: gamma amplitude is increased for the duration of the movement (event-related synchronization, ERS; (Pfurtscheller & Lopes da Silva, 1999; Miller *et al.*, 2009; Muthukumaraswamy, 2010); these ERSs occur even when proprioceptive feedback is absent (Butorina *et al.*, 2014). Gamma activity does not change before movement onset, during passive movements or movement observation (Muthukumaraswamy, 2010; Cheyne & Ferrari, 2013). These findings support a pro-kinetic role for gamma activity, as it seems to be related to active movement production, but not motor planning or sensorimotor processing.

Whether gamma activity better encodes kinematic or kinetic parameters is still under debate. ECoG studies from non-human primates report that high gamma power (70 – 110 Hz) encodes grasp force and muscle activity (Shin *et al.*, 2012; Chen *et al.*, 2014), but more recent ECoG patient studies indicate that high gamma activity may encode kinematics, such as grasp type, higher-order joint angle, start and end of the movement, regardless of the levels of force produced (Pistohl *et al.*, 2012; Flint *et al.*, 2017; Branco *et al.*, 2019*b*;

Jiang *et al.*, 2020). These conflicting results might arise from small sample sizes in both patient and non-human primate studies, variability in electrode placements, or overlap between kinetic and kinematics parameters during movement execution. Better understanding how to optimally decode movement parameters from gamma-band recordings would significantly improve the performance of BMIs, allowing better use of prosthetics for patients with impaired motor functions (Branco *et al.*, 2019*a*).

Modulating gamma activity can lead to changes in motor behaviour. Gamma-tACS over the left M1 increased the speed of voluntary force generation (Joundi *et al.*, 2012; Moisa *et al.*, 2016) and improved performance on a visuomotor task when sudden motor reprogramming was required, the effect being more pronounced with stimulation at high-gamma frequency (80 Hz; (Santarnecchi *et al.*, 2017)). The tACS-induced changes in neural activity were correlated with tACS-induced changes in motor performance (Moisa *et al.*, 2016). Some studies however show no effect of M1 gamma-tACS on motor performance (Miyaguchi *et al.*, 2018; Guerra *et al.*, 2018). When administered to experienced athletes, gamma-tACS did not significantly change their motor performance post-stimulation (Giustiniani *et al.*, 2021). It is possible that, since they had substantial motor training, they had already achieved a ceiling effect on their performance or that the effects of gamma-tACS did not persist after stimulation. High-gamma-tACS, but not low-gamma-tACS, was associated with improvements in motor learning (Sugata *et al.*, 2018; Giustiniani *et al.*, 2019), indicating different roles for the two frequency sub-bands.

1.2.4 Theta oscillations

Theta activity is characterised by oscillatory activity between 4 – 8 Hz and its role in hippocampal-based memory and learning has been widely studied (Buzsáki & Moser, 2013; Lisman & Jensen, 2013). Evidence from animal studies shows that theta activity can modulate both the firing patterns of single neurons and gamma power in neuronal population (Bragin *et al.*, 1995; Lee *et al.*, 2005; Lopes-Dos-Santos *et al.*, 2018).

In humans, the theta phase is coupled with gamma power: the largest high gamma amplitude occurs at the most excitatory phase of the theta waveform and increasing theta power strengthens this coupling (Canolty *et al.*, 2006). Theta-gamma coupling has been reported in both short-term and long-term memory tasks, increased coupling generally being associated with better task performance (Lega *et al.*, 2016; Alekseichuk *et al.*, 2016; Vivekananda *et al.*, 2021). Low and high gamma frequencies coupled with theta activity at different phases, which should inform future brain stimulation studies (Vivekananda *et al.*, 2021).

This phase-amplitude coupling (PAC) has been proposed as a mechanism for cortical computations and network connectivity (Fries, 2009). Patterns of coupling are reproducible for similar behavioural tasks, suggesting that PAC can modulate network activity for optimal task performance (Canolty *et al.*, 2006). Modulating PAC using brain stimulation may therefore change performance on motor tasks. Indeed, M1 stimulation with a tACS theta-gamma peak protocol (gamma frequency stimulation delivered during the peak of a theta envelope) lead to improved motor learning during stimulation, which persisted at 75 minutes post-stimulation (Akkad *et al.*, 2021). Whether this brain

stimulation protocol could also enhance motor rehabilitation in clinical populations remains to be investigated.

1.2.5 Technical considerations for tACS studies

Transcranial alternating current stimulation (tACS) is one of the widely used techniques in studying the role of brain oscillations. However, the results of tACS studies are not always consistent and several key factors might contribute to this variability. First, the stimulation protocols are not standardised; not only the duration, intensity and phase of tACS may differ, but also the size of the electrodes and the montage, which ultimately affect the strength of the generated electric field (Orendáčová & Kvašňák, 2021). Second, the effect of tACS may depend on participant-related factors. For example, people have individual peak frequencies for different frequency bands, yet tACS studies generally use fixed stimulation frequencies for all participants. Performing the stimulation at the participant's peak frequency may enhance the effects of the stimulation (Romei *et al.*, 2016). Additionally, the strength of the generated electric field will also depend on anatomical and physiological characteristics of the participants, such as age, head volume and shape, gyrification or myelination (Beliaeva *et al.*, 2021). Third, the effect of tACS on behaviour is usually quantified using simple, non-standardised tasks, which makes comparing results of different studies difficult (Gao *et al.*, 2020). While future studies should focus on replicating behavioural effects using stimulation parameters and tasks similar, it would also be interesting to investigate the role of tACS in more complex, naturalistic tasks. Lastly, the effect of tACS on cortical activity is not completely understood. tACS is thought to entrain neuronal populations; at single-neuron levels, tACS has been shown to affect the timing, but not the rate, of neuronal spiking (Krause *et*

al., 2019). However, others argue that tACS may induce weak electrical currents, incapable of entraining neuronal populations (Huang *et al.*, 2017; Lafon *et al.*, 2017; Lafleur *et al.*, 2021). Whether tACS-induced entrainment might be due to peripheral nerve stimulation is still under debate (Asamoah *et al.*, 2019; Krause *et al.*, 2019). For a recent review discussing the effect of tACS on neuronal oscillation, please see (Riddle & Frohlich, 2021).

1.3 THE SIGNIFICANCE OF MOTOR LEARNING IN HUMAN LIFE

Learning new motor skills contributes immensely to a healthy, fulfilling life. People acknowledge its importance at all ages: there are motor milestones that mark the physiological development of infants and toddlers; learning how to cycle or drive are celebrated achievements in the lives of children and young adults, which increase their independence; from trampolining to senior zumba, we learn new motor skills whenever we start a new hobby. As a society, we have come to appreciate how difficult it can be to achieve certain motor skills and applaud them in those talented; this is why we listen to concert pianists and watch artistic gymnastics.

Motor learning in healthy people can be used as a model for the recovery of motor functions after brain injury, like a stroke. Stroke is a leading cause of morbidity; stroke-induced disabilities significantly affect the survivors' independence and are projected to cost the UK £75 billion p.a. by 2035 (Hankey, 2017). Most of the spontaneous recovery of stroke survivors occurs within the first 6 months post-stroke and is thought to include motor learning mechanisms, but it is often incomplete (Krakauer, 2006). When associated with motor training, adjuvant therapies such as non-invasive brain stimulation might facilitate these mechanisms and lead to long-lasting improvements in motor outcomes for chronic stroke patients (more than 6 months post-stroke) (Allman *et al.*, 2016). Better understanding the mechanisms underlying motor learning in healthy people is our best strategy to design novel adjuvant therapies for motor rehabilitation and address this unmet need.

1.4 THE ROLE OF INHIBITION IN MOTOR LEARNING

Normal brain function relies on the tightly regulated balance between brain excitation and inhibition, processes mediated by excitatory and inhibitory neurotransmitters. Numerous studies have investigated the role of inhibition in motor learning and found a strong link between GABA, the most common inhibitory neurotransmitter in the adult mammalian brain, and motor plasticity and learning (Dayan & Cohen, 2011; Bachtiar & Stagg, 2014). Early mechanistic studies in rat neocortex show that application of GABA antagonists in M1 leads to induction of long-term potentiation (LTP; (Castro-Alamancos *et al.*, 1995)), while TMS studies in humans indicate that administration of GABA agonists leads to the suppression of LTP-like processes in M1 (McDonnell *et al.*, 2007). A recent study in our group has replicated a previous finding showing a decrease in [GABA] in M1 during motor sequence learning in humans, [GABA] being measured using Magnetic Resonance Spectroscopy (MRS) (Floyer-Lea *et al.*, 2006; Kolasinski *et al.*, 2019*a*). Moreover, decreases in M1 [GABA] induced through non-invasive brain stimulation (NIBS) correlate with better motor learning (Stagg *et al.*, 2011*a*).

These findings are consistent with evidence from stroke survivors, where GABA plays a key role in motor rehabilitation (Grigoras & Stagg, 2021). Several human MRS studies have reported a decrease in [GABA] in the ipsilesional hemisphere at rest in chronic stroke survivors (Głodzik-Sobańska *et al.*, 2004; Blicher *et al.*, 2015; Mooney *et al.*, 2019), with lower inhibition being associated with better hand function (Ferreiro de Andrade & Conforto, 2018). Functional improvements following rehabilitation interventions have also been associated with changes in inhibition. The relative improvement in function after 2 weeks of constraint-induced movement therapy (CIMT) was positively correlated

with decreases in MRS-derived [GABA] in ipsilesional M1 (Blicher *et al.*, 2015). Moreover, baseline [GABA] predicted subsequent response to a non-invasive brain stimulation intervention (O'Shea *et al.*, 2014).

All the evidence above indicates that decreased inhibition is associated with better motor learning outcomes. However, findings from human research are generally only correlational due to the nature of behavioural and neuroimaging techniques, as well as the limited availability of mechanism-specific interventions.

1.5 AIMS OF THIS THESIS

In this thesis, I aim to use pharmacological interventions in order to target specific GABA receptors and investigate how increasing GABA-mediated inhibition impacts human motor learning.

First, I present the results from a double-blind, randomised, placebo-controlled study in young, healthy volunteers, where I assess the effect of baclofen, a specific $GABA_B$-receptor agonist, on motor learning, brain chemistry and brain activity. Specifically, in Chapter 3, I discuss the effect of baclofen on motor behaviour, motor learning and the concentration of GABA in motor areas. In Chapter 4, I report the effect of baclofen on patterns of brain activity during the performance of a motor sequence task. In Chapter 5, I discuss the effect of baclofen on patterns of brain activity at rest and the relationship between metrics of functional connectivity and [GABA] in the sensorimotor network.

In Chapter 6, I present the results from a second double-blind, randomised, placebo-controlled study, where I studied the effect of zolpidem, a specific $GABA_A$-receptor positive allosteric modulator, on motor learning and electrophysiological measurements of GABA-mediated inhibition.

Lastly, in chapter 7, I summarise the main findings from the two pharmacological studies, discuss their main limitations and present avenues for future research.

CHAPTER 2 - METHODS

In this chapter, I will describe the methods used in my thesis to investigate the role of inhibition in human motor learning. First, I will discuss behavioural tasks to study motor control and motor learning, and the functional network underlying behavioural performance for each task. I will then discuss functional MRI, used to investigate brain activity during task performance and at rest, and Magnetic Resonance Spectroscopic Imaging (MRSI), used to measure the concentration of neurochemicals in a region of interest. Lastly, I will discuss transcranial magnetic stimulation (TMS), which was used to measure corticospinal excitability and GABA-mediated inhibition. For each technique, I will describe its mechanism of action, usage in research and limitations.

2.1 BEHAVIOURAL TASKS EVALUATING MOTOR CONTROL AND MOTOR LEARNING

Better understanding motor learning mechanisms is crucial for determining novel therapy options to aid motor rehabilitation in clinical populations. To investigate these physiological mechanisms in humans, research studies employ several behavioural tasks that probe different aspects of motor control and motor learning. In the following chapters, I used the action selection task to evaluate motor execution, and serial reaction time task and visuomotor adaptation to evaluate motor learning.

2.1.1 Action Selection Task

The action selection task has two separate instructions (execute and select), which help distinguish between motor execution and motor action selection (O'Shea *et al.*, 2007). During execute blocks, participants are instructed to press the same button for each trial within a block, regardless of the visual stimulus, thus evaluating motor execution by measuring response times to visual cues. On select blocks, participants are instructed to follow a more complex rule, where visual stimuli corresponds to different buttons, so the participants need to select the correct action based on the shape and size of the visual stimulus. The time necessary for the selection of the correct motor action can be measured by subtracting the reaction time on execute blocks (motor execution time) from the entire reaction time on select blocks (motor execution time + action selection time). Appropriate behaviour for each instruction type is characterized by the activity of different networks: motor execution relies on the sensorimotor network, including both cortical (primary motor areas, premotor areas, supplementary motor areas) and subcortical (basal ganglia) areas, while selecting the correct action (select – execute) relies on the action selection network, which is a bilateral premotor-parietal network, with spatially enlarged activity in the left hemisphere (Rushworth *et al.*, 1998, 2003; Grafton *et al.*, 1998; Schumacher *et al.*, 2003; O'Shea *et al.*, 2007). The activity of the left premotor cortex (PMd) seems to play a key role, as impaired performance on the task is associated with greater activation of the left PMd and decreased M1-PMd connectivity (Stewart *et al.*, 2014). This is particularly important for clinical populations, where the premotor cortex contributes to their motor performance post-injury and modulating its activity might improve action selection during motor rehabilitation (Johansen-Berg *et al.*, 2002; Stewart *et al.*, 2020). In the following chapters, we used the AS task as a non-learnable

motor task to compare motor execution and action selection between treatment conditions; we can then use this information when trying to explain any effects of drugs on motor learning, where motor learning, or motor execution, or action selection, or all three, might be improved.

2.1.2 Serial Reaction Time Task (SRTT)

Numerous activities in everyday life consist of a set of actions that only achieve the goal when executed in a certain order; this set of actions can consist of discrete movements, such as locking the door (reach and grab the key, insert the key in the lock, turn the key, take the key out) or it can be a continuous longer movement, such as swimming strokes or performing a tennis serve. Therefore, understanding how people learn to execute movements sequentially is an important part of better understanding motor learning.

The serial reaction time task (SRTT) has been widely used to evaluate motor sequence learning by training participants to perform simple movements (e.g. pressing a button) in a specific order, much like learning a very short piano piece (Robertson, 2007; Krakauer *et al.*, 2019). The order of the movements is shown through sequential visual stimuli, each mapped to a specific movement (Robertson, 2007; Johnstone *et al.*, 2021*b*). As participants learn which movement in the sequence comes next, their reaction times decrease, indicating better learning. The SRTT usually also includes random blocks, where the stimuli are associated with the same movements, but they are presented in a random order. The difference between reaction times on random versus sequence blocks is therefore a measure of the degree of sequence learning since the mapping of the stimuli to the movements and the length of individual movements (button presses) should remain the same between blocks. However, there is an element of sequence-independent

improvement in task performance: response times tend to be faster on a second random block than the first, likely due to the practice of individual movements and task familiarisation (Wong *et al.*, 2015; Krakauer *et al.*, 2019).

It has been hypothesized that motor sequence learning relies on the formation of a higher-level representation of the sequence so that the sequence can be planned and executed from the presentation of the first stimulus; indeed, for fairly short sequences (3-5 buttons), the reaction time of the first movement is slower with more complex sequences (Henry & Rogers, 1960; Sternberg *et al.*, 1978; Harrington & York Haaland, 1987). Longer sequences are generally split into smaller chunks, the length of which are determined by working memory capacity; the start of each chunk is highlighted by a longer reaction time, likely the time to plan for the chunk to be executed (Sakai *et al.*, 2003; Bo & Seidler, 2009). Chunking can aid in learning future sequences, since participants perform rearranged-chunk sequences better than random sequences (Sakai *et al.*, 2003).

The location of this higher-level sequence representation is still under debate: one recent study found a sequence engram in the primary motor cortex (Hamano *et al.*, 2020), but others reported no learning-related changes in M1 activity, indicating that M1 activity only reflected the activity for the individual movements (Mushiake *et al.*, 1991; Yokoi *et al.*, 2018). Learning-related changes have been found in premotor and prefrontal areas, as well as the basal ganglia. The supplementary motor area (SMA) in particular has been linked to motor sequence learning, as it is active during the motor planning stage and its activity changes during learning (Tanji, 1996; Doyon *et al.*, 2002; Elsinger *et al.*, 2006). Moreover, disrupting SMA activity using repetitive transcranial magnetic stimulation

(rTMS) impairs motor sequence performance (Gerloff *et al.*, 1997). Learning-related increases in activity in the basal ganglia, specifically in the putamen, correlates with behavioural metrics of motor sequence learning; however, since patients with basal ganglia pathologies can still learn an explicit motor sequence, it is possible that the basal ganglia contribute either to implicit motor sequence learning or a motivational component to perform the task (Jueptner *et al.*, 1997; Doyon *et al.*, 1998; Vakil *et al.*, 2000; Ungerleider *et al.*, 2002; Muslimovic *et al.*, 2007). Knowing which brain areas are key for motor sequence learning is crucial for developing new adjuvant therapies for motor rehabilitation; modulating the activity of these key areas with non-invasive brain stimulation might enhance neurorehabilitation in clinical populations.

2.1.3 Visuomotor Adaptation (VMA) Task

In everyday life, people do not only learn new sequences of movements, but they also adjust their movements to adapt to changes in their environment. For example, a tennis player will adapt their movements to the characteristics of the playing field, weather conditions and weaknesses of the opposing player. Understanding the circuits involved in visuomotor adaptation may help us enhance the adaptability and flexibility of well-known movements to new conditions in clinical populations.

In research studies, adaptation has been studied using tasks which disturb the dynamics of the movement (e.g. force field tasks where forces are imposed to the hand during reaching movements, requiring the participants to adjust their movements accordingly in order to reach the target) or the visuomotor mapping (e.g. visuomotor adaptation task where a perturbation has been applied to the visual feedback of reaching movement, so participants need to adjust their movements to compensate for this perturbation)

(Krakauer *et al.*, 2000, 2019). In visuomotor adaptation tasks, participants are unaware of the perturbation and adjust to it throughout a number of trials, while they receive feedback about their movement trajectory and target error. The process of adaptation has been hypothesized to rely on implicit adaptation due to sensory prediction errors; when participants were told about the perturbation and instructed to aim to a different position than the target, they could immediately adapt to the perturbation by aiming at the new target, but this effect did not last and their performance worsened (Mazzoni & Krakauer, 2006; Taylor & Ivry, 2011; Schween *et al.*, 2014). Having adapted to the visuomotor perturbation, participants may show aftereffects: they can keep performing perturbation-adjusted movements even when visual feedback has been removed, although aftereffects are only temporary and participants return to their default sensorimotor mapping relatively quickly without visual feedback (Redding & Wallace, 1993; Kluzik *et al.*, 2008; Taylor & Ivry, 2011; Huang *et al.*, 2011). However, some longer-term effects on behaviour may remain if no active washout task is performed. Participants may show savings: they learn the perturbation faster when exposed to it a second time compared to the first (Krakauer *et al.*, 2005, 2019). A two-state model has been proposed for visuomotor adaptation: one "fast" component, which adapts and decays rapidly, likely related to task reward, and one "slow" component, which adapts slowly, retains the skill for longer and is likely related to sensory prediction (Smith *et al.*, 2006; McDougle *et al.*, 2015; Huberdeau *et al.*, 2015). This model could explain the presence of both aftereffects and savings, as the residual effect of the "slow" component. The "slow" and "fast" components are thought to involve different circuits. The cerebellum is key to predictive control and visuomotor adaptation (Shadmehr *et al.*, 2010; Sokolov *et al.*, 2017): higher activity in the cerebellum was detected on trials when a perturbation was present,

compared to no perturbation, and patients with cerebellar degeneration showed impairments in visuomotor adaptation (Martin *et al.*, 1996; Rabe *et al.*, 2009; Schlerf *et al.*, 2012, 2013). Meanwhile, the primary motor cortex is involved in the retention, but not adaptation, of visuomotor skills, as reported by studies using non-invasive brain stimulation (Richardson *et al.*, 2006; Galea *et al.*, 2011).

2.2 NEUROIMAGING

Magnetic Resonance Imaging (MRI) has been widely used to study the structure and function of the brain *in vivo* for several decades. In the following chapters, I used functional MRI (fMRI) to study brain activity during task performance and at rest, and Magnetic Resonance Spectroscopic Imaging (MRSI) to measure the concentration of different neurochemicals in a volume of interest.

2.2.1 Functional MRI

Functional MRI can be used to detect changes in blood flow to different brain areas, which reflect changes in brain activity. This is achieved due to the magnetic properties of hemoglobin: its deoxygenated form (deoxyhemoglobin) has higher magnetic susceptibility and creates magnetic moments, in contrast to its oxygenated form (oxyhemoglobin), which does not create magnetic moments (Pauling & Coryell, 1936; Cerdonio *et al.*, 1977). Therefore, when the oxygen is extracted from the blood and into the tissues, it leads to a change in the magnetic properties of the blood. Oxygenated blood and the surrounding tissues have a similar magnetic susceptibility; after oxygen extraction, the now deoxygenated blood will have higher magnetic susceptibility than the surrounding tissue, creating magnetic field distortions and decreasing the MR signal.

However, when the activity of a brain area increases (for example, in response to a stimulus), the blood flow to that area increases more than the oxygen consumption (Fox & Raichle, 1986); thus, there is a counterintuitive increase in oxyhemoglobin and decrease in deoxyhemoglobin, which leads to an overall increase in the MR signal. This blood oxygenation level-dependent (BOLD) signal has been first introduced in 1990 and has since become an extensively used tool in studying brain function (Ogawa *et al.*, 1990, 1992; Kwong *et al.*, 1992; Buxton, 2013). While it has several advantages (non-invasive, non-radiant, good spatial and temporal resolution), it also has some limitations, such as difficulties in imaging deep brain structures, difficulties in imaging clinical populations (decreased mobility and MRI safety), and high cost of data acquisition and storage.

In this thesis, I used task fMRI to investigate the changes in brain activity during performance of motor tasks, as reflected by the measured BOLD signal. For both the action selection and the serial reaction time task, participants performed the task inside the MRI scanner in a block design: 'on' blocks of task performance (button pressing) were alternated with 'off' rest blocks of similar length. Thus, the change in brain activity between the on and off blocks should reflect the increase in activity of brain areas responsible for task performance.

Then, I also used resting-state fMRI to investigate functional connectivity between different brain areas. Resting-state fMRI can be used to measure low-frequency fluctuations in the BOLD signal in order to determine the brain areas in which the activity follows similar temporal patterns.

For both task fMRI and resting-state fMRI, I used an echo-planar imaging (EPI) sequence, commonly utilised for acquiring fMRI data in short periods of time (Stehling *et al.*, 1991).

2.2.2 Magnetic Resonance Spectroscopic Imaging

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) is an MR technique which allows reliable and non-invasive quantification of neurochemicals *in vivo* (Stagg & Rothman, 2014). ^{1}H-MRS can provide measurements of neurotransmitters such as glutamate and GABA, but with limited spatial and temporal resolution: MRS quantifies the total amount of a neurochemical in a relatively large MRS voxel of interest (8-15cm^3) acquired over minutes.

MRS relies on the magnetic resonance effect, described by the *Larmor equation*:

$$\omega = \gamma B$$

where ω is the resonance frequency, γ is the gyromagnetic ratio (a nucleus-specific constant) and B is the strength of the magnetic field. When protons are excited by a magnetic pulse, they first become polarized with the magnetic field and then, when the pulse is shut off, their spins rotate along the axis of the magnet, creating a rotating magnetic field at the resonance frequency. Protons are the most commonly used MR-sensitive isotope, because most metabolites in the brain contain hydrogen, and its gyromagnetic ratio is higher than many other stable isotopes. When the proton is part of a molecule, there is a specific change in its resonance frequency due to shielding from the molecule's electrons, which is called the chemical shift. Since the chemical shift is molecule-dependent, this helps us determine the concentration of specific molecules, because the MRS resonance signal will be proportional to the concentration of the resonating nucleus. Since water is the predominant molecule in the brain, the signal for water is much larger than for other molecules and can hide the signal from the

neurochemicals of interest, so it is essential to perform water suppression on the MRS data (Nixon 2008).

Localising the volume of interest is most commonly done using two localisation sequences: Point RESolved Spectroscopy (PRESS; (Bottomley, 1987)) and STimulated Echo Acquisition Mode (STEAM; (Frahm *et al.*, 1987)). Localisation is achieved using three magnetic pulses with slice-selective gradients (one for each axis), so that the volume of interest is found at the intersection of these three slices. Other single-voxel MRS sequences include adiabatic implementations, such as Localisation by Adiabatic Selection refocusing (LASER) and semi-LASER, which have the advantages of being robust against radiofrequency amplification variations, less sensitive to magnetic field inhomogeneities and enhanced volume localisation (Garwood & DelaBarre, 2001; Scheenen *et al.*, 2008; Kaiser *et al.*, 2008).

Magnetic Resonance Spectroscopic Imaging (MRSI) is a technique with better spatial resolution than single-voxel MRS: a single-voxel MRS sequence localizes the volume of interest and then MRI techniques are used to subdivide it into smaller voxels (van der Graaf, 2010; Emir *et al.*, 2017; Chiew *et al.*, 2018; Steel *et al.*, 2018). To enable this increased spatial resolution, the acquisition of MRSI is more technically challenging: it requires better quality of B_0 shimming to compensate for magnetic field distortions, better water and lipid suppression and increased scan times (Koch *et al.*, 2007; Stagg & Rothman, 2014).

In this thesis, I used a semi-LASER MRSI sequence, where the k-space sampling was performed using density-weighted concentric ring trajectories (CRTs), as described previously (Steel *et al.*, 2018). The MRSI volume of interest (85 x 35 x 15 mm^3) was a grid

of 17 x 7 voxels, each 5 x 5 x 15 mm^3, which was placed bilaterally over the primary and premotor cortices (Fig. 2.1).

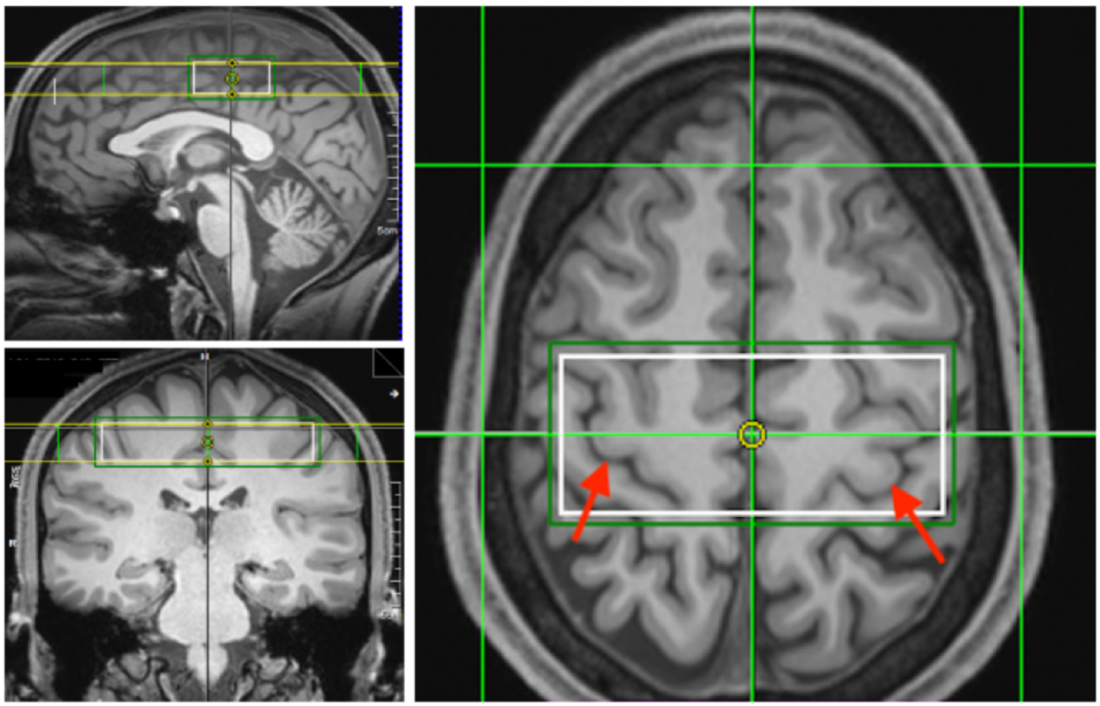


Fig. 2.1. **Placement of MRSI slab.** The MRSI slab was placed over the sensorimotor cortex, as to include the left and right-hand knobs (red arrows)

Therefore, I was able to measure the concentration of GABA in voxels of 375mm^3, much smaller than the single-voxel MRS, and used this data to study GABA and other neurochemicals between different regions of interest (primary motor cortex, premotor cortex, motor network). An example of the acquired spectra from one of the MRSI voxels is shown below (Fig. 2.2). Data preprocessing and fitting is described in more detail in the Methods section of Chapter 3.

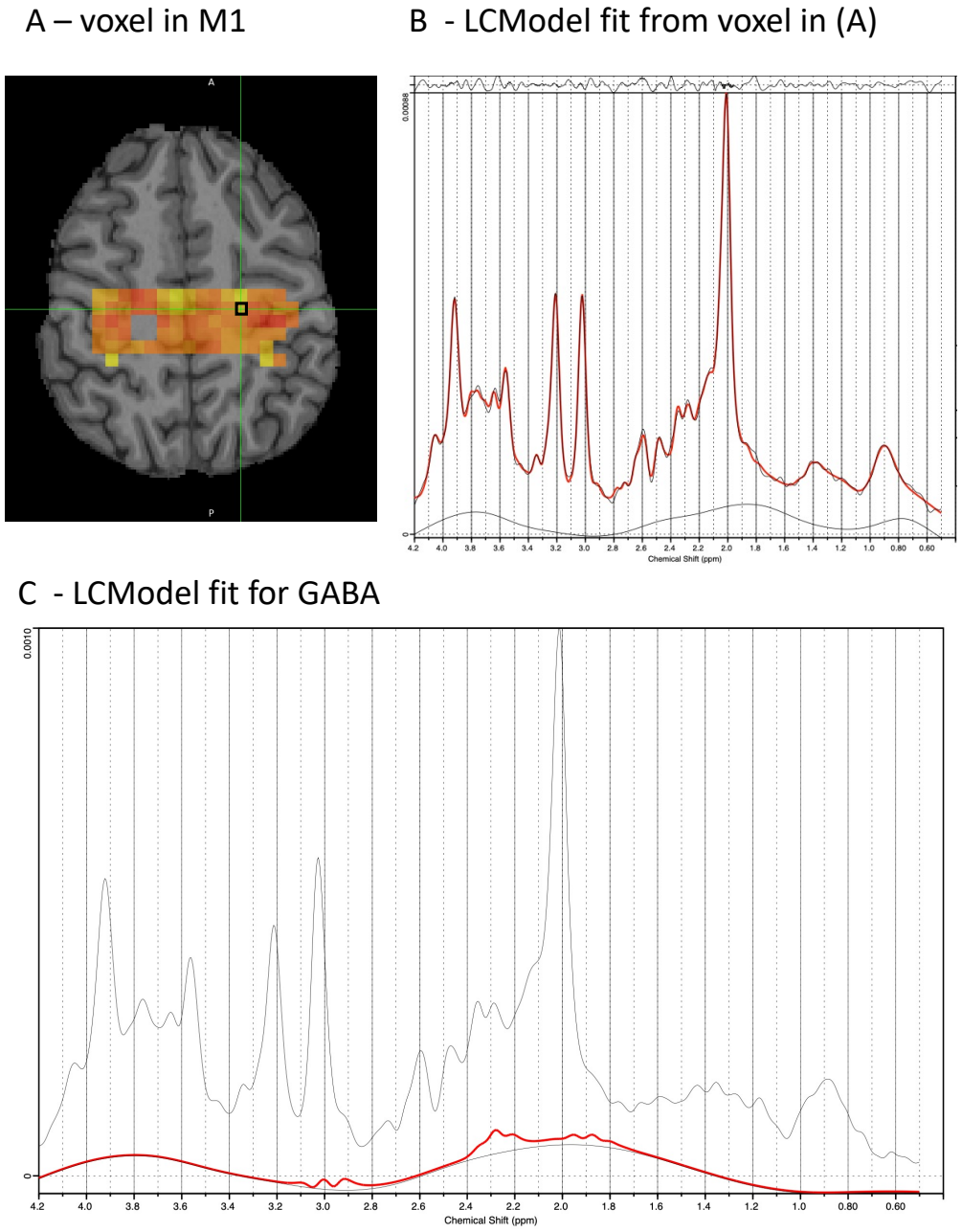


Fig. 2.2. **Example of ^{1}H-MRS spectra from one MRSI voxel.** (A) GABA/Cre map from one participant. The following LCModel fit is from the voxel highlighted in the black frame. (B) LCModel fitting output for data from voxel in (A). (C) LCModel fit for GABA

MRS-derived measurements of neurochemicals most closely reflect the total concentration in the volume of interest, but their relationship to electrophysiological measurements is still unclear. This MRS measurement of total concentration may

however be influenced by how much of the neurochemical is stored in intracellular vesicles. It has been proposed that neurochemicals bound to vesicular macromolecules are 'shielded' from the MR signal, being invisible to MRS, while the neurochemicals found extracellularly or in the cytosol are visible to MRS (Kauppinen & Williams, 1991; Jelen *et al.*, 2018; Lea-Carnall *et al.*, 2021).

Considering the timescale of the MRS acquisition (minutes), it is unlikely that the total neurotransmitter concentration is related to the synaptic activity; indeed, multimodal MRS and transcranial magnetic stimulation (TMS) studies consistently reported no relationship between MRS-derived GABA concentrations and TMS measurements of synaptic inhibition (Stagg *et al.*, 2011*b*; Tremblay *et al.*, 2013; Mooney *et al.*, 2017; Dyke *et al.*, 2017). A relationship has been proposed between [GABA] in M1 and what is believed to be a TMS measure of extrasynaptic inhibition (Stagg *et al.*, 2011*b*; Stagg, 2014), but this result has not been replicated in recent studies (Mooney *et al.*, 2017; Dyke *et al.*, 2017).

2.3 Transcranial Magnetic Stimulation (TMS)

TMS has been used extensively to investigate cortical excitability and intracortical inhibition in both healthy people and clinical populations (Talelli *et al.*, 2006; Burke *et al.*, 2019).

When using TMS to evaluate the physiology of the motor system, an electromagnetic coil is positioned on the scalp over the primary motor cortex (M1). When the coil discharges a magnetic pulse, this induces an electric current in M1 neurons through electromagnetic induction. If the current is large enough and if the neurons are appropriately oriented

compared to the direction of the induced current, this will cause neuronal depolarization and neuronal firing of the pyramidal cells in the motor cortex. The axons of these neurons are part of the corticospinal tract and synapse onto motoneurons in the spinal cord, leading to subsequent muscle contraction, which can be measured using electromyography (EMG) as motor-evoked potentials (MEPs) (Fig. 2.3). In this thesis, I used a DuoMAG MP-Paired Pulse TMS machine to collect electrophysiological measurements by recording muscle activity from the first dorsal interosseous muscle (FDI) in the right hand of young, healthy participants.

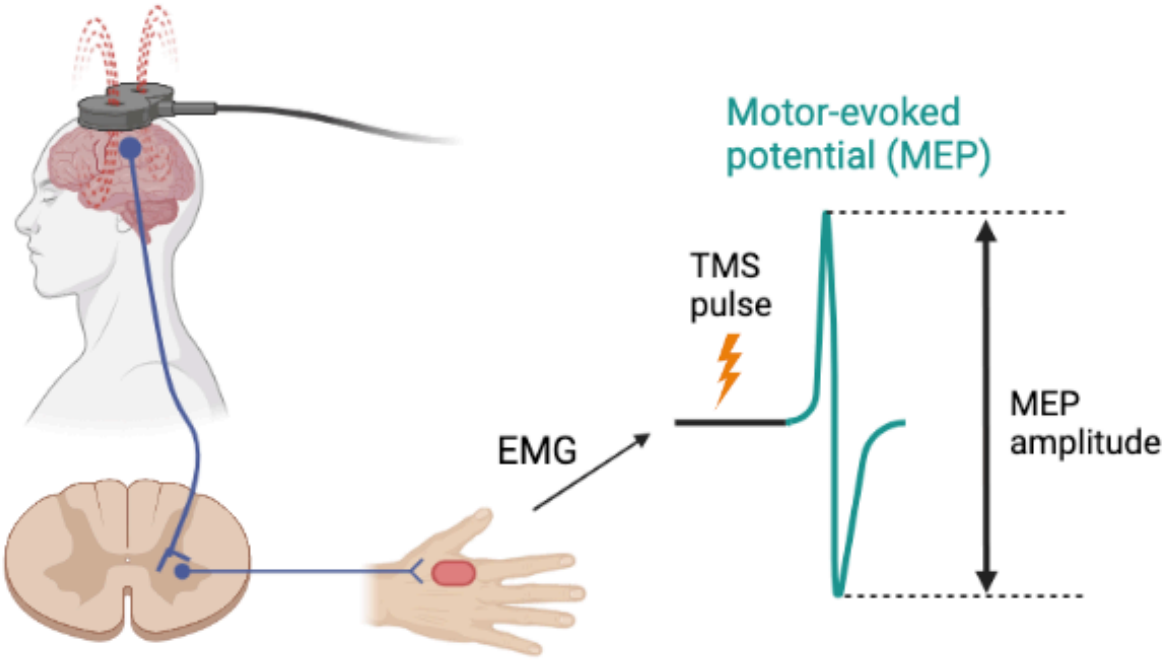


Fig. 2.3. **Schematic of TMS mechanism of action and detection of MEPs using electromyography (EMG)**. Figure created in bioRender

2.3.1 Single-pulse TMS

Single-pulse TMS provides a measurement of overall corticospinal excitability, as MEP amplitude increases with stimulus intensity following a sigmoid function (Devanne 1997). If higher stimulation intensity is required to achieve a certain amplitude of the MEP, that is thought to reflect lower excitability of the motor cortex and spinal cord structures (Di Lazzaro *et al.*, 2008; Rosso & Lamy, 2018). However, other factors also impact the stimulation intensity required to elicit MEPs, such as inter-individual variability in the distance between the cortex and the coil influenced by skull thickness, cerebrospinal fluid and brain anatomy, and intra-individual variability determined by changes in circadian rhythm, sleep and physiological noise (McConnell *et al.*, 2001; Wassermann, 2002; Rossini *et al.*, 2015). Pharmacological interventions can also affect the amplitude of MEP elicited by single-pulse TMS: administration of $GABA_A$ receptor positive modulators or dopamine agonist cabergoline tends to decrease the MEP amplitude, while administration of norepinephrine agonists and some selective serotonin reuptake inhibitors increase the MEP amplitude (Ziemann, 2021).

In this thesis, I used single-pulse TMS to determine the 1mV resting motor threshold (rMT), the minimum stimulation intensity required to elicit a 1mV MEP in 5 out of 10 consecutive trials at rest. I then determined the active motor threshold (aMT), the minimum stimulation intensity required to elicit a 0.2mV MEP in 5 out of 10 consecutive trials when participants were contracting the FDI muscle at approximately 30% of their maximum contraction (Rossini *et al.*, 2015). I also collected single-pulse TMS measurements during the paired-pulse TMS protocols, as described below.

2.3.2 Paired-pulse TMS

Paired-pulse TMS uses two pulses at different interstimulus intervals (ISIs) to provide measurements of neurotransmission mediated by specific neurochemicals, including GABA. The first pulse is called *the conditioning pulse* and is thought to activate cortical interneurons which modulate the activity of pyramidal neurons; the second, supra-threshold pulse is called the test pulse and activates pyramidal neurons leading to muscle activity, as described in single-pulse TMS. Since different interneuron populations have different activation and deactivation times, modulating the ISIs can help investigate the activity of different types of receptors on the pyramidal neurons. Inhibition mediated by the two main classes of GABA receptors, $GABA_A$ and $GABA_B$, can be at least mostly distinguished using quite different stimulus paradigms.

Short-interval intra-cortical inhibition (SICI) can be measured with an ISI of 1-5ms and is thought to reflect $GABA_A$ receptor activity (Fisher *et al.*, 2002), as administration of $GABA_A$ receptor agonists increases SICI (Ziemann *et al.*, 1996*a*; Di Lazzaro *et al.*, 2000; Ilić *et al.*, 2002; Di Lazzaro *et al.*, 2005; Ziemann, 2021).

Long-interval intra-cortical inhibition (LICI) can be measured with an ISI of 50-200ms and is thought to reflect GABAB receptor activity, as administration of $GABA_B$ receptor agonists increases LICI (McDonnell *et al.*, 2006).

In this thesis, I used ISIs of 1ms and 2.5ms to record the SICI measurements and ISIs of 100 and 150ms to record LICI. For each protocol, 12 pulses were delivered in each condition (single pulse, first ISI, second ISI) in a pseudorandomized order. The ratio between the MEPs recorded in each paired-pulses condition and the single pulse

condition represents the change in muscle activity determined by the conditioning pulse; smaller numbers would indicate more inhibition.

2.3.3 Limitations of TMS

Even if TMS measurements are widely used to study excitability and inhibition, TMS remains a noisy technique (Beaulieu *et al.*, 2017). To get a robust measurement, the mean of a large number of measurements is calculated to eliminate noise that might arise from oscillatory activity in the motor cortex (Cavaleri *et al.*, 2017; Nowak *et al.*, 2017); in this study, I used 12 pulses per condition, but other studies have used fewer. Whether participants have their eyes opened or closed can also influence MEP sizes (Chen & Huang, 2018), which is why I asked participants to keep their eyes open during collection of TMS data. The quality of the TMS measurements is also dependent on the experimenter's proficiency, which cannot be assessed from the data. Neuronavigation systems can help guide coil placement, but I chose not to use neuronavigation in the project presented in Chapter 6 because participants were too uncomfortable to wear the sensors on a headband for the whole duration of the sessions (2 x 5 hours). Finally, acquiring TMS measurements in clinical populations is difficult, as corticospinal tract damage reduces MEPs; this leads to a bias in TMS studies towards patients with mild and moderate motor impairments.

2.4 SUMMARY

This thesis aims to investigate the role of inhibition in human motor learning using pharmacological interventions targeting specific GABA receptors. This section has described the methods used to measure motor behaviour and learning, and to study brain activity at rest and during motor learning. Behavioural tasks were employed to study motor control and motor learning, with distinct tasks looking at motor sequence learning versus visuomotor adaptation. Task fMRI was used to look at patterns of brain activity during motor sequence learning, while resting-state fMRI to look at functional connectivity at rest. MRSI was used to quantify the concentration of neurochemicals in a volume of interest. Lastly, TMS was used to measure corticospinal excitability and GABA-mediated inhibition.

CHAPTER 3 - THE EFFECT OF BACLOFEN ON MOTOR LEARNING AND MRS-DERIVED [GABA] IN MOTOR AREAS

Motor learning plays a significant part in our lives. Whenever we reach and grab the fork to eat, cycle to work or play frisbee with our friends, we use previously learnt motor skills. Understanding the neurophysiological changes underlying healthy motor learning may not only help us to understand our daily lives, but it may also aid the search for new adjuvant therapies for people re-learning motor skills after brain injury such as stroke. There is a close link between the levels of inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the primary motor cortex (M1), and motor plasticity and learning (Floyer-Lea *et al.*, 2006; Stagg *et al.*, 2011*a*; Kolasinski *et al.*, 2019*a*). *Modulating GABAergic inhibition may therefore affect how people learn new movements, with important implications for motor rehabilitation after stroke. Here, I used baclofen, a $GABA_B$ receptor agonist, as a pharmacological intervention to test the hypothesis that pharmacologically increasing GABAergic inhibition would impair motor learning in young, healthy volunteers. This question is clinically relevant: baclofen is commonly prescribed to stroke survivors as a muscle relaxant, which might impair their motor rehabilitation.*

3.1 INTRODUCTION

The balance between excitatory and inhibitory neurotransmitters is crucial for normal brain function. Increasing evidence has linked GABA-mediated inhibition and motor plasticity and learning *(Dayan & Cohen, 2011; Bachtiar & Stagg, 2014)*, a relationship which has been described in more detail in Chapter 1. Notably, the concentration of GABA in the primary motor cortex (M1) decreases during motor sequence learning (Floyer-Lea

et al., 2006; Kolasinski *et al.*, 2019*b*) and decreases in M1 [GABA] induced by non-invasive brain stimulation correlate with better motor learning (Stagg *et al.*, 2011*a*). However, the causality of this relationship is still unclear, due to the correlational nature of the techniques employed in human research and the paucity of mechanism-specific interventions. Receptor-specific pharmacological interventions may help bridge this knowledge gap. Here, I used GABA-specific drugs in young healthy volunteers to answer the question of whether increasing inhibition may lead to impairments in motor learning. This is a clinically relevant question since GABAergic agonists are commonly used in clinical practice as antiepileptics, hypnotics or muscle relaxants and they might negatively impact the motor rehabilitation of stroke survivors receiving them.

3.1.1 Baclofen and motor learning

Baclofen is a specific $GABA_B$ receptor agonist commonly used in clinical practice as a muscle relaxant (Simon & Yelnik, 2010; Draulans *et al.*, 2013). By activating the metabotropic GABA receptor on the postsynaptic site, baclofen increases potassium conductance via G protein-activated K^+ channels (GIRK), leading to hyperpolarisation of the postsynaptic cell (Newberry & Nicoll, 1984, 1985). On the presynaptic site, baclofen-activated $GABA_B$ receptors inhibit both voltage-gated and P/Q type ($Ca_v2.1$) Ca^{2+} channels and decrease neurotransmitter release (Benarroch, 2012). $GABA_B$ receptors show high expression in areas related to motor control, such as the frontal cortex, thalamic nuclei and cerebellum; in rodent globus pallidus, their expression even surpasses that of ionotropic $GABA_A$ receptors (Bowery *et al.*, 1987).

When administered orally to humans, baclofen crosses the blood-brain barrier; it reaches peak plasma concentration in approximately 1-2 hours and has a half-life of 3-6 hours (Ziemann *et al.*, 1996*a*). In clinical practice, baclofen is used in patients experiencing muscle spasticity post-stroke or multiple sclerosis. Patients usually receive doses of 5-10 mg multiple times throughout the day, depending on the severity of their symptoms.

Two previous studies have reported impairments in performance on a visuomotor adaptation task after a single dose of 10-20mg baclofen (Willerslev-Olsen *et al.*, 2011; Johnstone *et al.*, 2021*b*). However, the neurochemical mechanisms underlying this behavioural change are still unclear. Both studies used TMS to collect neurophysiological measurements of GABA-mediated inhibition: (Willerslev-Olsen *et al.*, 2011) only collected single pulses measuring corticospinal excitability, while (Johnstone *et al.*, 2021*b*) had a more complex TMS dataset, including both single- and paired-pulse protocols that measure $GABA_A$ (SICI) and $GABA_B$ (LICI) receptor activity. Both studies reported decreased corticospinal excitability after baclofen administration. However, (Johnstone *et al.*, 2021*b*) did not find a significant effect of baclofen on either $GABA_A$- or $GABA_B$-mediated inhibition to explain the behavioural effects, i.e. impaired visuomotor retention. To better understand the mechanisms mediating this behavioural effect, I aim to investigate the role of baclofen on motor learning and brain chemistry using neuroimaging techniques. This could change clinical guidance for baclofen use in patients undergoing motor rehabilitation.

3.1.2 Aim

To investigate the role of baclofen on motor learning and brain chemistry, I conducted a within-subject, double-blind, placebo-controlled study where participants had an MRI scan 45 minutes after administration of a single dose of 20mg of baclofen or placebo. During the scan, I acquired MRSI data before and after motor learning to obtain concentrations of neurochemicals in M1 at these time points. I also acquired task fMRI data during motor learning alongside behavioural data, to study the mechanisms underlying the baclofen-related changes in motor learning. **I hypothesised that baclofen would significantly impair motor learning and that this behavioural change would be associated with changes in M1 [GABA] during learning.**

3.2 METHODS

3.2.1 Participants

I recruited 18 healthy participants who took part in this study by giving their informed consent to all experimental procedures, as approved by the Central University Research Ethics Committee (Ethics reference: R55534/RE004). All participants met the following inclusion criteria: aged 18-35 years (mean age +/- SEM 24.3 +/- 4.1 years, 8 males), right-handed as per the Edinburgh Inventory (Oldfield, 1971), no history of any psychiatric or neurological illness, not taking any medication, not musically trained (not more than Grade 6 on the Associated Board of the Royal Schools of Music) and 3T MRI safety criteria.

3.2.2 Experimental design

I conducted a within-subject, double-blind, placebo-controlled study to investigate how increasing GABAergic inhibition via baclofen, a $GABA_B$ receptor agonist, affected motor learning and brain chemistry. Participants attended two sessions at least one week apart, starting at the same time of day, as GABA levels fluctuate during the circadian rhythm (Cardinali & Golombek, 1998). The experimental design for the two sessions was identical (Fig. 3.1), except participants received a single dose of 20mg of baclofen during one session and a placebo pill (sucrose/lactose) on the other. Both participant and experimenter were blinded to the treatment condition on each session, as the encapsulation and randomisation for this study were completed by Dr Liliana Capitao at the NIHR Oxford Health Biomedical Research Centre.

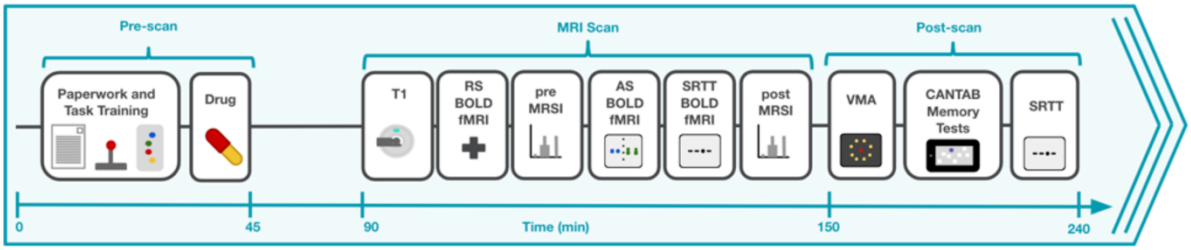


Fig. 3.1 **Timeline of the testing session**. The pre-scan period consisted of initial paperwork and sensorimotor task training: MRI-safety screening, Edinburgh handedness test, mood questionnaires and task training (action selection [AS], serial reaction time task [SRTT], and visuomotor adaptation [VMA]). During the MRI scan period, participants were first asked again to fill out the MQs at 45 min post-drug administration (PA). Participants then entered the MRI scanner for ~1 hr, during which we recorded: T1 structural scan, resting-state (RS) functional MRI (fMRI), pre-motor-task magnetic resonance spectroscopy imaging (MRSI), fMRI with simultaneous AS task, fMRI with simultaneous SRTT, and post-motor-task MRSI. During the post-scan period, participants filled in the third set of MQs (2h PA), VMA task, CANTAB memory tests, and a shortened version of the SRTT. Adapted from MSc student thesis (Geist 2019)

On arrival, participants completed a mood questionnaire, specifically the Bond Lader Visual Analog Scale (BLVAS), and were familiarised with 3 motor tasks: the action selection task (AS), the serial reaction time task (SRTT) and the visuomotor adaptation task (VMA).

Next, participants received the treatment (baclofen or placebo) and had a 45-minute break to allow for baclofen to be absorbed and reach peak plasma concentration (Ziemann *et al.*, 1996*a*; Agarwal *et al.*, 2015). After the break, the participants filled in a BLVAS questionnaire again followed by an MRI scan. The scanning time was approximately 1 hour, starting with a T1-weighted structural scan, resting-state functional MRI (fMRI) and pre-task MRSI. Participants then completed the AS and SRTT tasks with simultaneous fMRI recordings for each task. Lastly, a post-task MRSI sequence was run.

After the MRI scan, participants filled in a third BLVAS questionnaire 2 hours after treatment administration, completed a 20-minute VMA task and a battery of Cambridge Cognition (CANTAB) memory tests.

3.2.3 Sensorimotor tasks

3.2.3.1 Action Selection Task (AS)

I used the action selection task (AS) as a non-learning motor task, to elicit information about how quickly participants executed and selected movements (O'Shea *et al.*, 2007). I familiarised the participants with a shorter version of the task before the MRI scan and then they completed the full-length task inside the scanner at approximately 1.5 hours after treatment administration. The duration of the task was 6 minutes 20 seconds and was completed with a simultaneous fMRI acquisition.

Participants were instructed to use their right index finger to press button 1 and their right middle finger to press button 2 on an MRI-compatible 4-button box. The AS task was composed of two types of blocks: Execute blocks (EBs) and Select blocks (SBs), each containing 15 trials. During execute blocks, participants were instructed to press the button listed in the instruction screen for each cue they saw (Fig. 3.2a). During select blocks, they were told to press button 1 if they saw a small square or a big circle and press button 2 if they saw a small circle or a large square (Fig. 3.2b). In total, the AS task had 9 blocks which were completed in a fixed order (Fig. 3.2c). One participant was excluded from further analysis because they did not correctly understand the task instructions and did not perform the execute blocks appropriately.

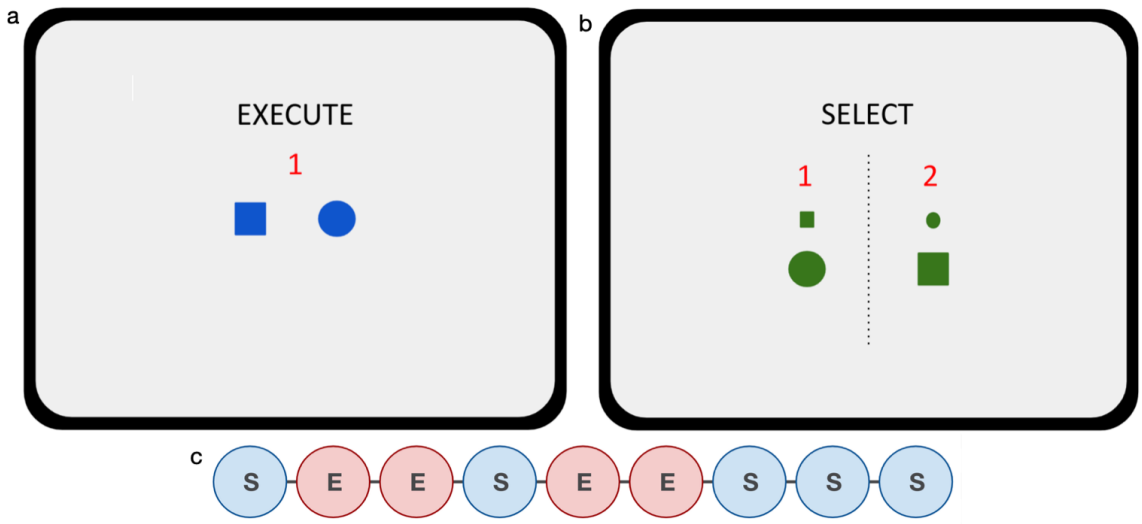


Fig. 3.2. **AS instructions and block order**. a) Sample EB instructions; b) Sample SB instructions; c) Block order

I calculated response times (RTs) for execute and select blocks as the time between stimulus presentation and the button press. I excluded incorrect button presses, pre-emptive button presses (RT < 50ms) and outliers (± 2.7 SD from the mean of each block type). I then calculated the median RT for each block type for each participant: the median RT for execute blocks was considered the participants' response time, and the difference between the median RT of select and execute blocks (SB – EB) was considered the participants' action-selection time.

3.2.3.2 Serial Reaction Time Task (SRTT)

To measure motor sequence learning, I asked participants to complete a serial reaction time task, adapted from a previous study (Robertson *et al.*, 2004).

Participants were instructed to use their right index, middle, ring, and little fingers to press buttons 1-4 on an MRI-compatible 4-button box in response to a visual cue, as quickly and accurately as possible. Participants were also instructed to not press a button before the visual cue appeared on the screen.

At the beginning of each block, participants would see a screen with four dashes (Fig. 3.3a). One of the dashes would then be replaced by an asterisk (Fig. 3.3b), which represented the visual stimulus for the participant to press the corresponding button. The SRTT was composed of two types of blocks: sequence blocks and random blocks, each with 60 trials repeated at a frequency of 1.5Hz. During random blocks, the order of the visual cues was random, so the participants could not anticipate which button would come next. During each sequence block in a session, the same 12-button sequence was repeated five times. Normally, as participants repeat the sequence, their RTs decrease, which is an indicator of motor learning (Robertson, 2007). Two different sequences were used for the two sessions. In total, the SRTT task had 15 blocks (2 of which were random), which were completed in a fixed order (Fig. 3.3c).

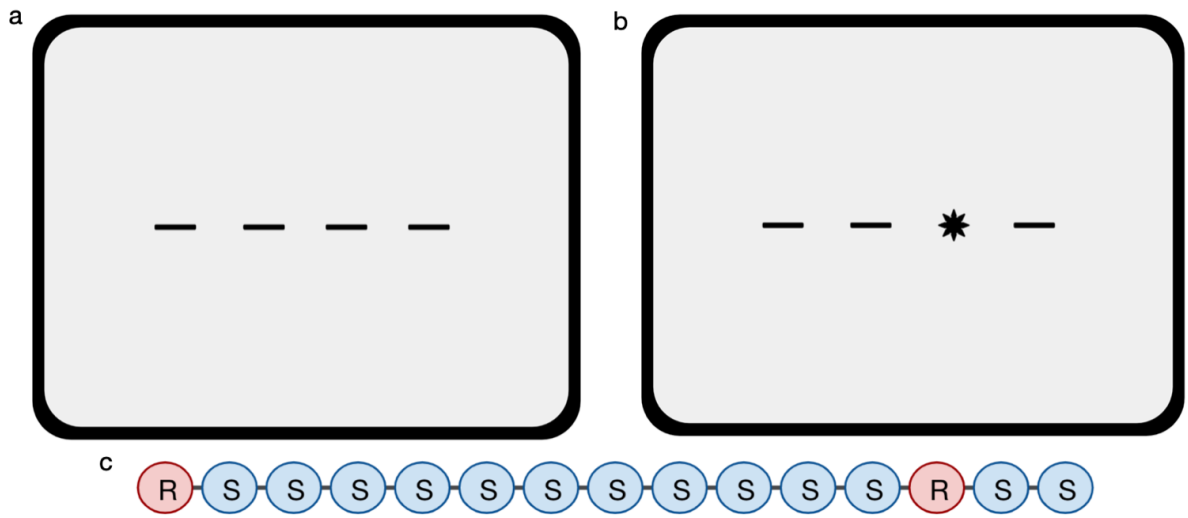


Fig. 3.3. **Serial reaction time task (SRTT)**. a) Rest screen; b) Sample cue; c) Block order

I calculated response times (RTs) for each block as the time between stimulus presentation and the button press. I excluded incorrect button presses, pre-emptive button presses (RT < 50ms) and outliers (± 2.7 SD from the mean of each block). I then calculated the median RT for each block and normalised it by dividing it by the median RT of the first random block (R1). I also calculated a learning gradient for each session by fitting a linear regression model to the data in sequence blocks 1 to 11. One participant was excluded from further analysis because their learning gradient on one session was classified as an outlier (± than 2.7 SD from the mean of all participants' learning gradients).

3.2.3.3 Visuomotor adaptation task (VMA)

To measure the participants' ability to adapt to perturbations of visuomotor mapping, I asked participants to complete a visuomotor adaptation task with similar instructions as previous studies (Galea *et al.*, 2011). Before drug administration, I familiarised the participants with a shorter version of the task which did not include any perturbation. They then completed the full-length 20-minute task at approximately 2.5 hours after treatment administration.

Participants used their right hand to move a joystick (Logitech Attack 3), which controlled a cursor on the computer screen (Dell Latitude E5540). For the entire length of the task, the participant's right hand was covered by a shielding box to prevent visual feedback of the hand's movements.

At the beginning of each trial, the joystick would be in the neutral position, placing the red cursor in the centre of the screen (Fig. 3.4a). For each trial, participants were instructed to make quick shooting movements towards the yellow target as accurately as possible, but without adjusting their trajectory during the trial.

The task was composed of two types of blocks: feedback-on blocks (FB-ON) and feedback-off blocks (FB-OFF). During FB-ON blocks, there was a 30° counterclockwise perturbation between the participant's hand movement and the trajectory of the cursor on the screen, so participants would see a 30°-deviated trajectory (Fig. 3.4b). This was unknown to the participants, who then had to adjust their hand movements to compensate for the perturbation. During FB-OFF, the cursor disappeared as soon as the participants moved the joystick. The lack of visual feedback allowed us to measure if participants retained the

30° perturbation to visuomotor mapping. In total, the task had 10 blocks, with alternating FB-ON and FB-OFF blocks. Each block contained five epochs; each epoch contained 8 consecutive trials, one for each defined target (Fig. 3.4a).

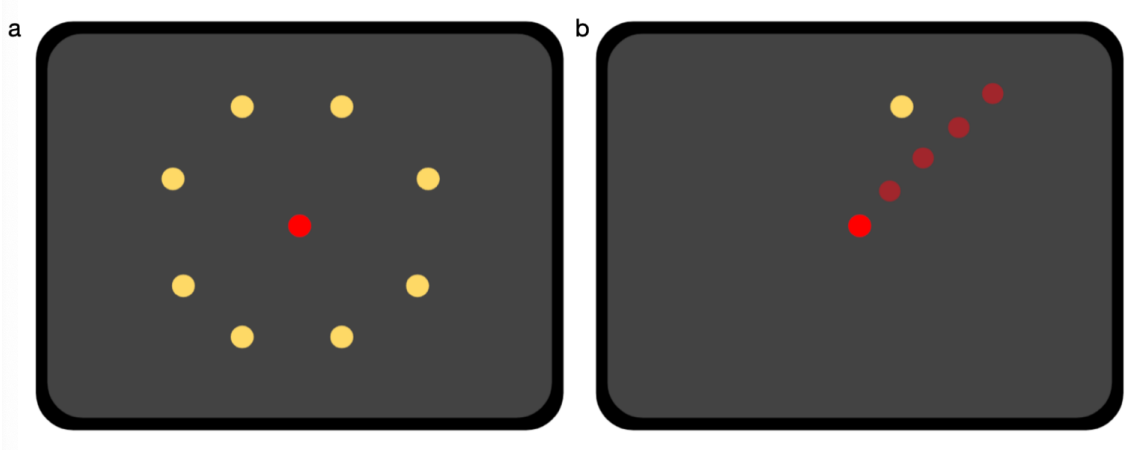


Fig. 3.4. **Visuomotor adaptation task (VMA).** a) Target circle: 8 potential targets (yellow) and cursor (red); b) Cursor trajectory in FB-ON blocks

I calculated the angular error for each trial as the angle between the target location and the point where the cursor crossed the imaginary target circle. On the trials where the cursor trajectory did not reach the imaginary target circle, the angular error was calculated at the point where the cursor crossed a circle with the radius half of that of the target circle. The trial was excluded if any of the following three conditions applied: cursor trajectory not reaching the half-radius point, angular error over 67.5° or the joystick not being in the neutral position at the start of the trial.

I then calculated the mean angular error for each epoch for each participant and used it for the statistical analysis. Six participants were excluded from the VMA analysis due to joystick malfunctions.

3.2.4 MR Data Acquisition

As previously described (Fig. 3.1), participants had an MRI scan in a 3T Siemens Magnetom System (PRISMA) with a 32-channel head coil (Nova Medical Inc., Wilmington, USA) that started 45 minutes after treatment administration. Three participants could not complete the MRI scans due to claustrophobia and were therefore not included in the neuroimaging analysis.

I acquired the following scans in this fixed order: T1-weighted structural MRI data (MPRAGE sequence, 1mm isotropic, TR = 1.9 s, TE = 3.96 ms, TA = 7.3 min, field of view 256 mm, flip angle 8°), resting-state fMRI (matched to the UK Biobank parameters for resting-state fMRI scan (Alfaro-Almagro *et al.*, 2018), pre-task MRSI (semi-LASER DW-CRT sequence with metabolite cycling (Steel *et al.*, 2018), TR = 1.4s, TE = 32ms, TA = 2 x 4.5min, voxel size = 5 x 5 x 15 mm^3), AS task fMRI (EPI, TR = 933ms, TE = 33.40ms, TA = 6.3 min), SRTT task fMRI (EPI, TR = 933ms, TE = 33.40ms, TA = 15.3 min) and post-task MRSI (as above). Both the resting-state fMRI and task fMRI analysis and results are discussed in the following chapter.

3.2.4.1 MRSI Data Acquisition

The MRSI slab (85 x 35 x 15 mm^3) was manually placed over both the left- and right-hand knobs at the posterior margin of the precentral gyrus (Kolasinski *et al.*, 2017), excluding the dura to minimise contamination due to macromolecules (Fig. 2.1) (Steel *et al.*, 2018).

Reconstruction of MRSI data and neurochemical quantification was performed using in-house scripts and LCModel, similar to that in previous studies (Provencher, 2001; Steel *et*

al., 2018; Nettekoven *et al.*, 2022). After metabolite cycling reconstruction (Emir *et al.*, 2017) and coil-combination (Walsh *et al.*, 2000), I corrected for frequency and phase shifts, removed residual water using HLSVD (Cabanes *et al.*, 2001) and finally corrected for Eddy currents (Klose, 1990). For quantification of neurochemicals, I used a chemical shift of 0.5 to 4.2ppm, 26 metabolites as a basis set, default LCModel macromolecules, no soft constraints on metabolites and a baseline stiffness setting (DKMTMN) of 0.25. The two MRSI acquisitions correspond to different time points; thus, I obtained metabolite maps both before and after the motor learning task.

From these metabolite maps, I only included in further analysis voxels that had a metabolite Cramer-Rao lower band (CRLB) < 50% and signal-to-noise ratio (SNR) > 40 (Fig. 3.5A). Metabolite concentrations are reported as the ratio of the respective metabolite to that of total creatine (creatine + phosphocreatine; tCr).

To confirm the optimal placement of the MRSI slab in all participants, metabolite maps were registered to MNI space using linear and non-linear registration (FLINT and FNIRT, (Jenkinson & Smith, 2001; Jenkinson *et al.*, 2002*a*)). All MRSI slabs correctly covered the hand area in the left hemisphere (Fig. 3.5B).

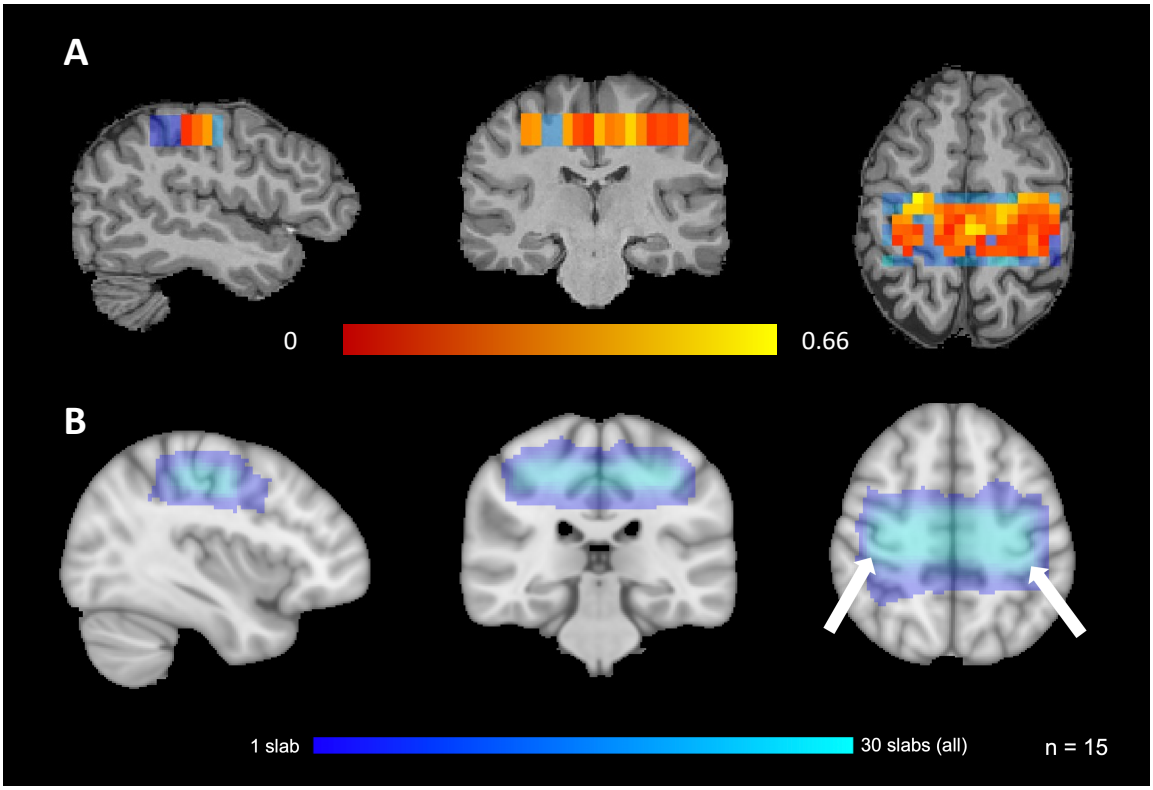


Fig. 3.5. **MRSI slabs.** (A) Example of GABA map in one participant – voxels that pass quality checks (SNR > 40, CRLB < 50%) in red and excluded voxels in blue. (B) All acquired MRSI slabs cover the left- and right-hand knobs (white arrows)

To quantify GABA in the brain regions responsible for motor control, I registered anatomical MNI maps of left and right M1 and premotor cortex to each participant's structural scan using FLIRT and FNIRT (Jenkinson & Smith, 2001). For each participant, I only included the voxels that passed quality control (Cramer-Rao lower band (CRLB) < 50% and signal-to-noise ratio (SNR) > 40) and were in the anatomical maps for each region of interest; I then calculated the mean metabolite concentration within these voxels for each participant. The resulting value was then used in further analysis.

3.2.5 Mood questionnaires

To quantify any changes in mood after baclofen administration, I asked participants to fill in the BLVAS three times during each session: before treatment administration, at 1 hour after treatment administration (just before the MRI scan) and 2.5 hours after treatment

administration (after the MRI scan). Analysis and results quantified from the mood questionnaire will be presented in the next chapter.

3.2.6 Working memory tests

To investigate the effect of baclofen on cognitive functions, I asked participants to complete four working memory tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) on a tablet (Apple iPad Air 2) at approximately 3 hours after treatment administration (Falconer *et al.*, 2010). These four tests were: Spatial Working Memory (SWM), Pattern Recognition Memory (PRM), Spatial Span (SSP), and Rapid Visual Processing (RVP). Further details on the 4 working memory tests, as well as data analysis and results, will be presented in the next chapter.

3.2.7 Statistical Analysis

All behavioural data were analysed using MATLAB (v. R2020b, MathWorks) and Prism (v.9.3.0, GraphPad Software). MRSI data were analysed using Bash scripts, R (RCoreTeam 2013) and Prism. Analysis was performed using repeated-measures ANOVAs with Geisser-Greenhouse's correction or paired t-tests with significance levels of $\alpha = 0.05$ to investigate the within-subject effects of baclofen compared to placebo. Relationships between MRSI and behavioural metrics were investigated calculating Pearson's correlation coefficients. All graphs show mean ± SD unless otherwise indicated.

3.3 RESULTS

3.3.1 Baclofen did not significantly affect motor performance

First, I investigated whether baclofen affected motor performance. I used two paired t-tests: one to detect if baclofen affected how quickly participants pressed a button in response to a visual stimulus (response times on execute blocks) and another one to detect the effect on baclofen on how quickly participants decided which button to press (action selection times). I found that baclofen did not significantly affect either response times on execute blocks ($t(13)$ = 0.5073, p = 0.621; Fig. 3.6A) or action selection times ($t(13)$ = 2.083, p = 0.058; Fig. 3.6B), indicating that baclofen did not significantly affect motor performance.

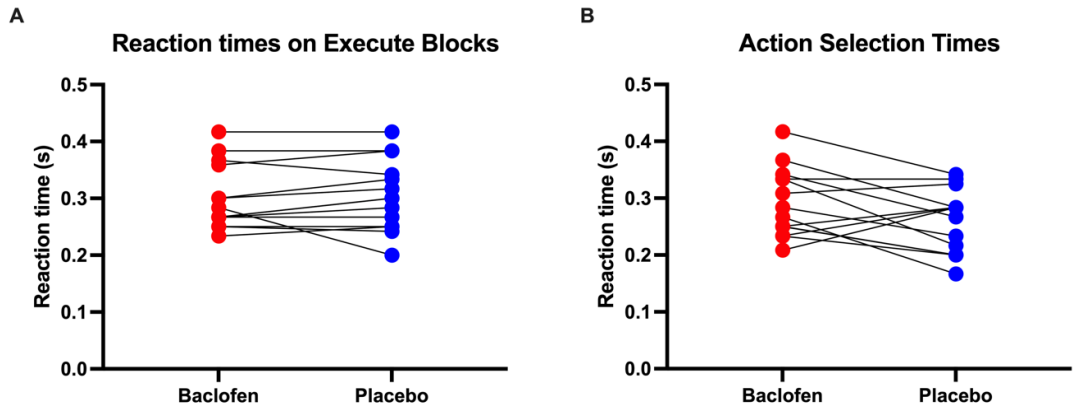


Fig. 3.6. **Behaviour on Action Selection Task.** No significant differences between baclofen and placebo sessions for response times on execute blocks (A) or action selection times (B)

3.3.2 Baclofen significantly impaired performance on a motor sequence learning task

I then investigated whether baclofen significantly impaired performance on the serial reaction time task.

Response times in the first random block (R1) were not significantly different between baclofen and placebo sessions (paired t-test: $t(13) = 0.161$, $p = 0.875$). I then normalised the median response times for each sequence block (S1 to S11) to the corresponding participant's R1 response time and used these normalised response times in the following analysis (Fig. 3.7).

Using a two-way repeated-measures ANOVA with within-subject factors of treatment (baclofen, placebo) and learning (S1, S2,… S11), I found a significant effect of treatment ($F(1,13) = 6.07$, $p = 0.029$) and a significant effect of learning ($F(3.179, 41.33) = 9.261$, $p < 0.001$), but no significant treatment x learning interaction ($F(4.568, 59.38) = 1.089$, $p = 0.373$). This would indicate that, while participants learnt the sequence on both sessions, response times during motor sequence learning were significantly slower on the baclofen session compared to placebo.

I then calculated the skill participants acquired during motor sequence learning, by subtracting the response times at the end of sequence learning (sequence block 11) from the response times on random block 2 (R2 – S11). I compared the motor skill acquired between treatment conditions using a paired t-test and found no significant difference between the baclofen and placebo sessions ($t(13) = 1.445$, $p = 0.172$).

I also investigated a potential effect of order using a two-way repeated-measures ANOVA with within-subject factors of session (session 1, session 2) and learning (S1, S2,… S11). I found a significant effect of learning ($F(3.179, 41.33) = 9.261$, $p < 0.0001$), but no significant effect of session ($F(1, 13) = 0.675$, $p = 0.426$) and no significant session x learning interaction ($F(4.284, 55.69) = 0.536$, $p = 0.721$), indicating there were no carry-over effects between sessions.

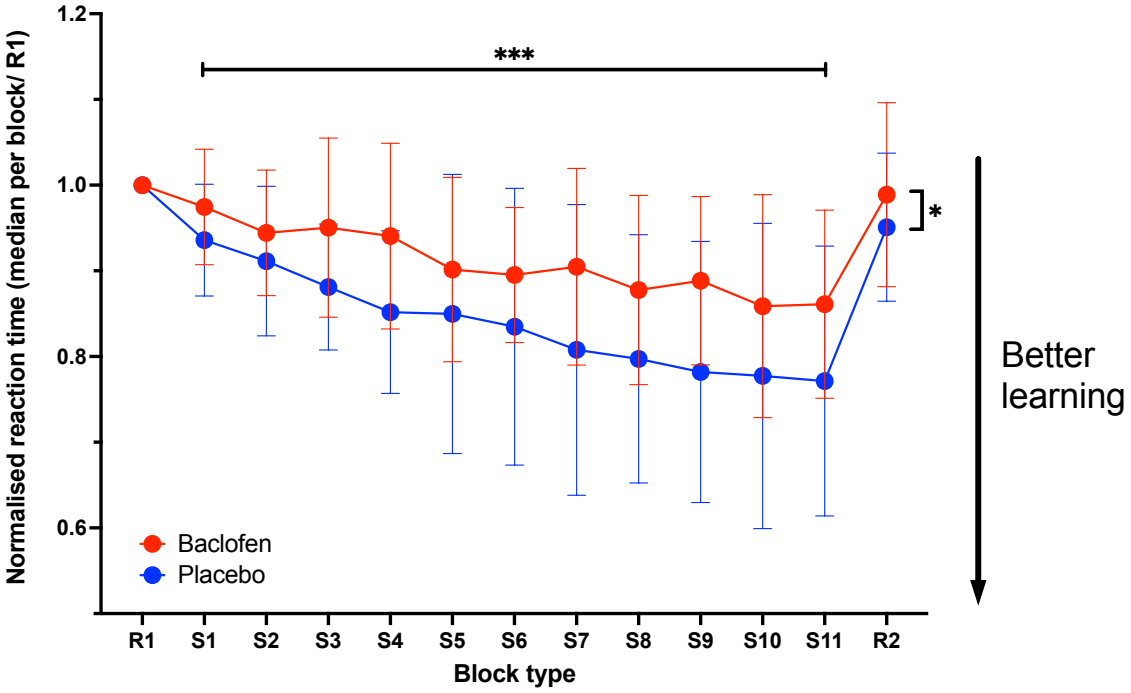


Fig. 3.7. **Behaviour on the Serial Reaction Time Task.** Motor sequence learning was significantly impaired after baclofen administration compared to placebo

3.3.3 Baclofen did not significantly affect visuomotor learning

I next investigated whether baclofen affected visuomotor adaptation and retention. As described above, I measured the mean angular error between the target presented on the screen and the participants' trajectory during both feedback-on and feedback-off blocks. The mean angular error for each of the 5 epochs within each block was used in a three-way repeated-measures ANOVA with within-subject factors of treatment (baclofen, placebo), block (B1, …, B5) and feedback (FB-ON, FB-OFF) (Fig. 3.8).

I found a significant effect of block ($F(3.301, 36.31) = 14.29$, $p < 0.0001$,) and feedback ($F(0.515, 5.665) = 1497$, $p < 0.0001$,), but no significant effect of treatment ($F(0.676, 7.425) = 0.178$, $p = 0.586$), no significant block x treatment ($F(4, 44) = 1.467$, $p = 0.228$) or block x treatment x feedback interactions ($F(1.513, 16.65) = 1.455$, $p = 0.257$). This would indicate that while participants learnt to adapt their movements to compensate for the perturbation, baclofen did not significantly affect this process.

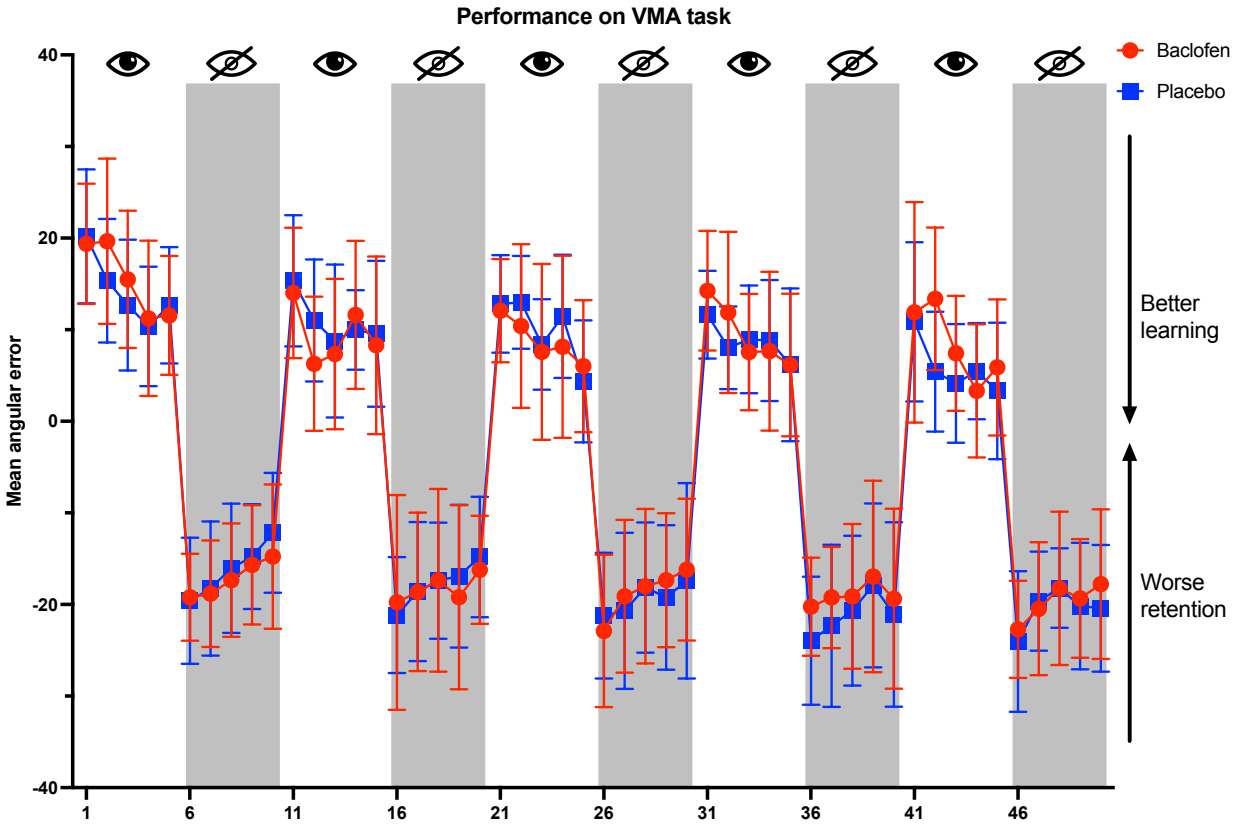


Fig. 3.8. **Behaviour on the visuomotor adaptation task.** No significant difference in mean angular errors during feedback-on blocks (white background) and feedback-off blocks (grey background) between baclofen and placebo session

3.3.4 Baclofen did not significantly change pre-task [GABA] in the left primary motor cortex and premotor cortex

To investigate how baclofen changed [GABA] in brain areas related to motor control, I first calculated [GABA] in the left M1 and left premotor cortex before motor learning (pre-task [GABA]; Fig. 3.9). I compared these values between baclofen and placebo conditions using paired t-tests and found no significant difference in pre-task [GABA] after baclofen administration in either of the brain regions (primary motor cortex: $t(14) = 1.915$, $p = 0.0761$; premotor cortex: $t(14) = 0.946$, $p = 0.360$; Fig. 3.10).

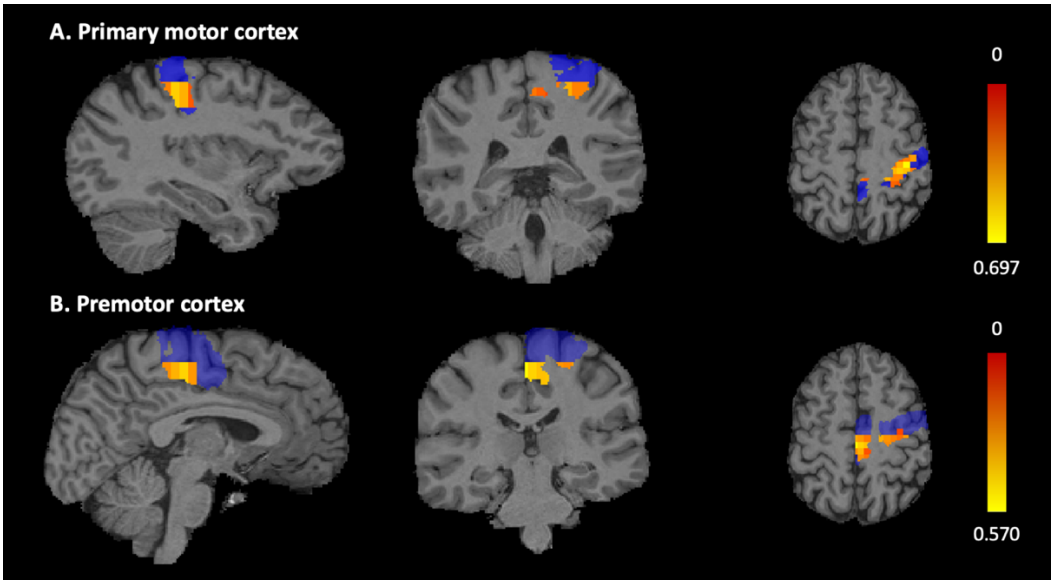


Fig. 3.9. **Examples of GABA/tCr concentrations** in voxels in the left primary motor cortex (A) and left premotor cortex (B). The anatomical mask for each brain region is shown in blue

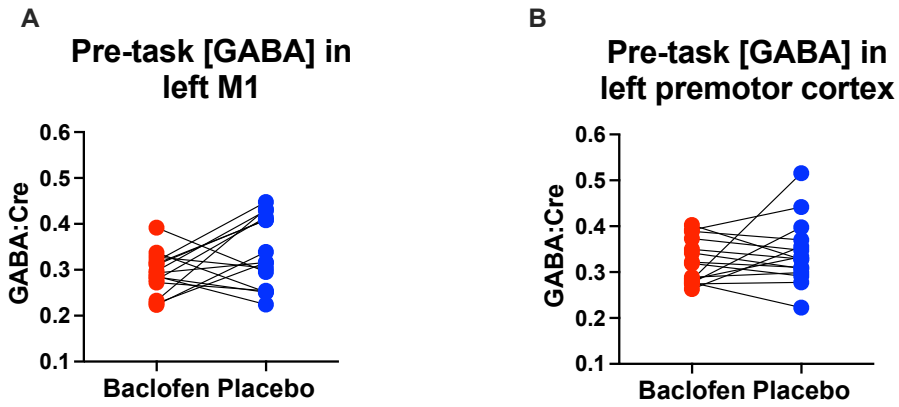


Fig. 3.10. **Pre-task [GABA]** did not significantly change in the left primary motor cortex (A) and left premotor cortex (B) after baclofen administration

3.3.5 Baclofen significantly affected the pattern of GABA change during motor learning

Inside the scanner, participants performed a motor sequence learning task using their right hand. I measured [GABA] both before and after the motor learning task in the brain regions responsible for motor control of the right hand: the left M1 and left premotor cortex. I then investigated how baclofen affected the pattern of [GABA] change during motor learning in these regions. I used a three-way repeated-measured ANOVA with factors of treatment (baclofen, placebo), learning (pre-task, post-task) and region of interest (ROI; left M1, left premotor cortex) (Fig. 3.11). I found a significant effect of ROI ($F(0.873, 11.34) = 8.128$; $p = 0.018$) and a significant treatment x learning interaction ($F(1, 13) = 5.908$; $p = 0.030$), but no significant effect of treatment ($F(0.907, 11.80) = 0.497$; $p = 0.477$), learning ($F(0.770, 10.01) = 0.92$; $p = 0.331$), ROI x treatment interaction ($F(1, 13) = 2.381$; $p = 0.147$), ROI x learning ($F(1, 13) = 2.207$; $p = 0.161$), or ROI x treatment x learning ($F(0.755, 9.819) = 0.558$; $p = 0.425$). This indicates that there was a significant difference in GABA levels between ROIs and that the [GABA] dynamics during motor learning were significantly different between baclofen and placebo sessions.

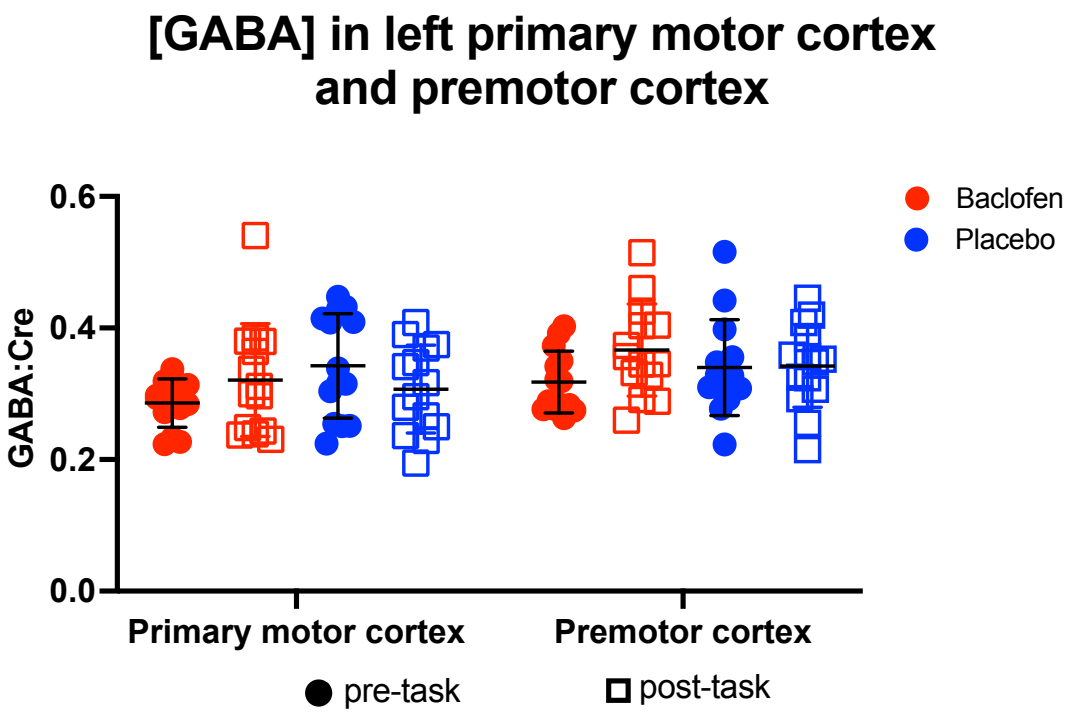


Fig. 3.11. **[GABA] before and after motor learning task**. Baclofen significantly affected the pattern of [GABA] change during motor learning

This finding is anatomically-specific: it was not replicated for the right M1 and right premotor cortex using a mixed-effects model with within-subject fixed-effects of treatment (baclofen, placebo), learning (pre-task, post-task) and ROI (right primary motor cortex, right premotor cortex). I found a significant effect of learning ($F(0.942, 12.24) = 5.129$; $p = 0.044$), but no significant effect of treatment ($F(0.719, 9.352) = 0.016$; $p = 0.828$) or ROI (; $F(0.973, 12.65) = 0.791$; $p = 0.387$) and no significant interactions: ROI x treatment ($F(1, 13) = 0.235$; $p = 0.636$), treatment x learning ($F(1, 13) = 0.026$; $p = 0.874$), ROI x learning ($F(1,13) = 0.548$; $p = 0.472$) and ROI x treatment x learning ($F(0.787, 8.658) = 0.011$; $p = 0.869$).

3.3.6 MRSI-derived [GABA] measurements were significantly correlated with behavioural metrics

I then investigated how MRSI-derived GABA measurements related to behavioural metrics. The mean response times during the two random blocks of the SRTT task were significantly correlated with placebo pre-task [GABA] in the left premotor cortex (Fig. 3.12A; placebo: $r(13) = 0.539$, $p = 0.038$; baclofen: $r(13) = -0.230$, $p = 0.409$), but not with pre-task [GABA] in the left M1 (Fig. 3.12B; placebo: $r(13) = 0.274$, $p = 0.324$; baclofen: $r(13) = -0.089$, $p = 0.752$). Specifically, higher levels of [GABA] in the left premotor cortex were associated with slower response times to visual stimuli. I then used a Fisher r-to-z transformation to evaluate if these correlations were significantly different between treatment conditions and found a significant difference between baclofen and placebo in the left premotor cortex ($z = 2.05$, two-tailed $p = 0.040$), but not M1 ($z = 0.91$, two-tailed $p = 0.363$).

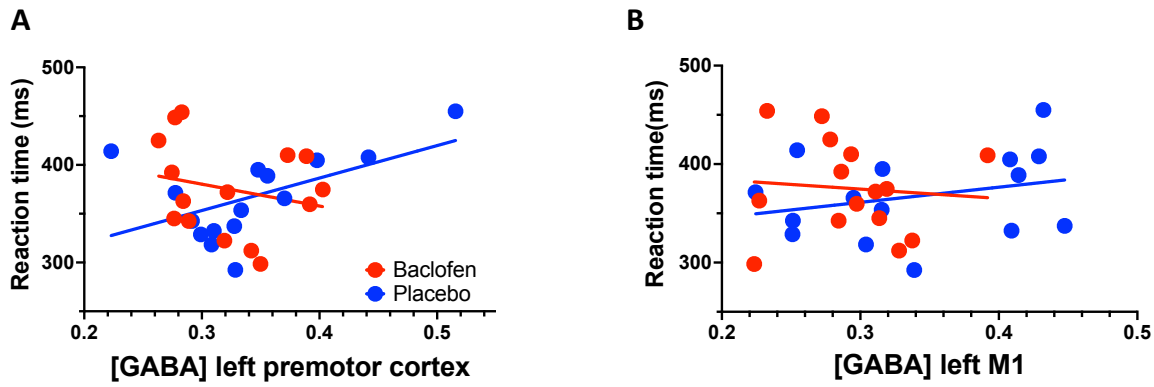


Fig. 3.12**. Relationship between pre-task [GABA] and response times during random button presses**. Significant correlation between pre-task [GABA] in the left premotor cortex (A) and response times during the placebo session. No significant correlation between pre-task [GABA] in the left M1 and response times (B)

I then studied how motor learning metrics related to [GABA] change during motor learning in brain areas responsible for motor control. As one motor learning metric, I used the difference in normalised median response times between the first and last sequence blocks [(S1-S11)/R1], so that higher values of this metric would represent larger decreases in response time during motor sequence learning and therefore, better learning. For the change in GABA during motor learning, I used the difference between the pre- and post-task [GABA], so higher values of this metric would represent larger decreases in GABA during motor learning.

I found a significant correlation between the learning metric and the Δ[GABA] in the left premotor cortex during the baclofen session, but not the placebo (Fig. 3.13A; baclofen: $r(12) = 0.579$, $p = 0.031$; placebo: $r(12) = -0.245$, $p = 0.399$). Specifically, higher decreases in premotor cortex [GABA] were associated with better motor learning. I did not find a significant correlation between the learning metric and the ΔGABA in the left M1 during either session (Fig. 3.13B; baclofen: $r(12) = -0.348$, $p = 0.223$; placebo: $r(12) = 0.288$, $p = 0.318$). I then used a Fisher r-to-z transformation to evaluate if these correlations were significant different between treatment conditions and found a significant difference between baclofen and placebo in the left premotor cortex ($z = 2.14$, two-tailed $p = 0.032$), but not M1 ($z = -1.55$, two-tailed $p = 0.12$).

Lastly, I used the skill acquired during motor learning (R2-S11) as another learning metric. I found no significant correlation between the skill and the Δ[GABA] in the left primary motor cortex in either session (baclofen: $r(12) = -0.287$, $p = 0.319$; placebo: $r(12) = 0.017$, $p = 0.953$), as well as no significant correlation between the skill and the Δ[GABA] in the left premotor cortex in either session (baclofen: $r(12) = 0.142$, $p = 0.629$; placebo: $r(12) = -0.418$, $p = 0.136$).

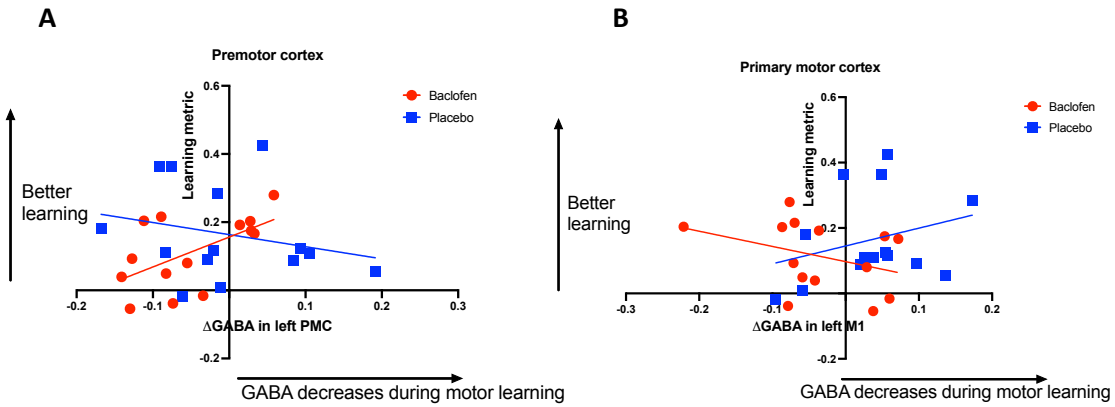


Fig. 3.13. **Relationship between Δ[GABA] and a learning metric** calculated based on performance during the SRTT

3.4 DISCUSSION

Here, I investigated the effect of $GABA_B$ receptor agonist baclofen on motor learning and brain chemistry. I found that increasing GABA-mediated inhibition *via* baclofen lead to significant impairments in performance on a motor sequence learning task, which were not associated with slower response times or slower action-selection times on the action selection task. I did not find a significant effect of baclofen on visuomotor adaptation; however, our sample size was much smaller for this task than the others due to joystick malfunctions. I also found that while baclofen did not significantly affect pre-task [GABA] in the left M1 and premotor cortex, it did significantly change the pattern of [GABA] change during motor learning in both these regions.

3.4.1 The effect of baclofen on behavioural metrics

3.4.1.1 Baclofen impairs performance on a motor sequence learning task

Since [GABA] decreases in M1 are associated with motor learning (Floyer-Lea *et al.*, 2006; Stagg *et al.*, 2011*a*; Kolasinski *et al.*, 2019*a*) and baclofen is known to suppress neuroplastic process in the motor cortex (McDonnell *et al.*, 2007), I set out to test whether increasing GABAergic inhibition using a pharmacological intervention would impair motor learning in healthy volunteers.

We found that baclofen significantly impaired performance on the serial reaction time task; specifically, response times were significantly slower on the baclofen session compared to placebo. Since there was no significant treatment x learning interaction, we cannot conclude a significant impairment in sequence learning, although we did not find slower response times with baclofen in the action selection task.

This finding contrasts with our previous study, which found no statistically significant effect of baclofen on motor sequence learning, although response times were visually slower on the baclofen session (Johnstone *et al.*, 2021*b*). Differences in dose and task design might account for this discrepancy. Impairments in motor learning may be dose-dependent and significant effects only appear with larger doses, as I administered a single dose of 20mg of baclofen to our participants compared to 10mg in Johnstone et al. There were also differences in task design: our SRTT had 11 blocks of sequence flanked by two random blocks, where participants would repeat the 12-button sequence 55 times in total. In Johnstone *et al*, the task had a familiarisation part with one sequence block flanked by two random blocks, which was followed by the training part consisting of 7 sequence blocks, where participants would repeat the 12-button sequence 28 times in total. It is possible that statistically significant learning decrements due to baclofen effects only appear with the longer training in our task. Moreover, the SRTT data analysis in Johnstone et al only measured skill gained at the end of the task (difference in RTs between last random and last sequence blocks), which would fail to consider how participants' RTs changed from one sequence block to the next and how this rate of motor learning changed between baclofen and placebo. Here, I addressed this by comparing the RTs in all sequence blocks between the two treatment conditions. Lastly, another notable difference is the SRTT pace: in Johnstone et al, the task was self-paced, while the visual cues in our task came at a fixed frequency (1.5Hz), due to constraints in the task-fMRI paradigm.

3.4.1.2 Baclofen did not significantly affect motor performance on the action selection task

I found no significant effect of baclofen on response times or action-selection times as measured using the AS task, which would suggest that the baclofen-mediated impairment of motor sequence learning was not a consequence of slower response time to visual cues or slower times to select the appropriate movement. This finding is consistent with a previous study that also did not find an effect of baclofen on simple response times, albeit at a lower dose of baclofen (Johnstone *et al.*, 2021*b*).

3.4.1.3 Baclofen did not significantly affect visuomotor adaptation

I also found no significant effect of baclofen on motor performance during a visuomotor adaptation task. However, two previous studies reported significant impairments in visuomotor adaptation and retention, as well as a significant positive correlation between TMS-derived metrics of $GABA_B$-mediated inhibition and worse retention during the VMA task (Willerslev-Olsen *et al.*, 2011; Johnstone *et al.*, 2021*b*). The absence of a baclofen-related significant effect in our study was likely due to technical difficulties. The joystick I first used malfunctioned in the first 6 participants, recording all movements made to the right side of the screen as vertical movements. Therefore, I could not use the VMA data from a third of the participants, limiting the power of our statistical analysis.

MRS-derived [GABA] in M1 has been reported to increase during audiomotor learning and this GABA increase correlated with better motor learning (van Vugt *et al.*, 2020), although TMS-metrics indicate an increase in corticospinal excitability after visuomotor adaptation (Bagce *et al.*, 2013). If I had a full VMA dataset, I would have aimed to also investigate whether behavioural metrics of adaptation and retention were significantly correlated

with MRSI-derived [GABA], recapitulating the previous finding between retention and TMS-derived $GABA_B$-mediated inhibition (Johnstone *et al.*, 2021*b*).

3.4.2 The effect of baclofen on MRSI-derived [GABA]

3.4.2.1 Baclofen did not significantly affect pre-task [GABA] in left M1 and premotor cortex

Pre-task [GABA] in both left M1 and premotor cortex was not significantly affected by baclofen administration. Baclofen is a specific $GABA_B$ receptor agonist which crosses the blood-brain barrier and increases GABA-mediated inhibition. However, MRSI-derived metrics of GABAergic inhibition do not directly reflect GABA receptors synaptic activity. In animal models, only one study in awake rodents acquired both neurophysiological data of excitatory and inhibitory activity through calcium imaging and MRS-derived measurements of GABA and glutamate from the barrel cortex. They found that, after whisker stimulation, the change in MRS-derived [GABA] and [glutamate] followed a similar direction to the change in inhibitory and excitatory activity, an effect that disappeared under anaesthesia (Takado *et al.*, 2022). In humans, there is substantial evidence showing no significant relationship between MRS-derived [GABA] and TMS measures of synaptic inhibition (Stagg *et al.*, 2011*b*; Tremblay *et al.*, 2013; Mooney *et al.*, 2017; Dyke *et al.*, 2017). Instead, MRSI provides measurements of the total amount of GABA within each MRSI voxel (375 mm^3), acquired over minutes. This MRSI-derived GABA measurement has been hypothesised to reflect extrasynaptic, tonic inhibition (Stagg *et al.*, 2011*b*; Stagg, 2014), although this is yet to be confirmed.

Baclofen does not have specificity over $GABA_B$ receptor subtypes, activating both pre-and post-synaptic $GABA_B$ receptors (Benarroch, 2012). On the presynaptic cell, baclofen binds to the $GABA_B$ receptor and decreases the quantity of neurotransmitter released into the synaptic cleft. On the postsynaptic site, baclofen binds to the $GABA_B$ receptor and leads to hyperpolarisation of the cell, but its binding to the receptors instead of the endogenous neurotransmitter could mean that GABA stays longer in the synaptic cleft until feedback mechanisms increase its reuptake into glial cells and neurons. The pathways of GABAergic signalling are complex and therefore the effect baclofen has on the total concentration of GABA and plasticity is difficult to predict; depending on the proportion of pre-and post-synaptic receptors that are activated by baclofen, total [GABA] may either decrease or increase (Mott *et al.*, 1990; Olpe & Karlsson, 1990; Ohliger-Frerking *et al.*, 2003; McDonnell *et al.*, 2006). Moreover, excitation-inhibition balance is a tightly regulated process, so it is perhaps unsurprising that, if baclofen significantly affected total [GABA] via changes in synaptic activity and neurotransmitter release, those effects would be short-lived and not be noticeable on a timescale lasting several minutes.

3.4.2.2 Baclofen significantly affected the pattern of [GABA] change during motor learning

Investigating how [GABA] changes during motor learning in the left M1 and premotor cortex, I found a significant effect of baclofen on the pattern of GABA change during motor sequence learning. In rats, one day of motor training is associated with decreased miniature inhibitory postsynaptic current frequency, suggesting a decrease in presynaptic GABA release after motor learning (Kida *et al.*, 2016). In humans, [GABA] in M1 is known to decrease during motor sequence learning (Floyer-Lea *et al.*, 2006; Kolasinski *et al.*,

2019*a*), but it is yet unknown how long this decrease lasts after the learning stops, although changes in GABA have been reported after long-term (6 weeks) of training (Sampaio-Baptista *et al.*, 2015). The reason I did not find a significant [GABA] decrease with learning in the placebo session may be that I acquired the data in the 9 minutes after completion of the motor learning task when [GABA] might have returned to normal. Another study has also reported no significant decreases in MRS [GABA] after motor learning. However, in this study, the sequence was executed with the participants' non-dominant hand and might be considered easier, with only 5 button presses (Maruyama *et al.*, 2021).

However, the significant treatment x learning interaction indicates that the effect of learning on [GABA] in the left M1 and premotor cortex is different between the baclofen and placebo sessions. Baclofen, therefore, seems to diminish the physiological M1 [GABA] decrease during motor learning, which was associated with impairments in performance on a motor sequence learning task. This is an important finding because it is further evidence that GABAergic signalling is important for normal motor learning and that modulating GABAergic inhibition can change the behavioural outcomes of motor learning tasks. Previous studies investigating how GABA changes during motor learning only show correlational evidence, but this study shows causal evidence that increasing GABAergic inhibition leads to impairments in performance on a sequence learning task.

I also found a significant effect of ROI on [GABA], meaning that there was a significant difference in [GABA] between the left M1 and premotor cortex. This is consistent with previous reports of MRS-derived [GABA] not being uniform across the cortex, with no

significant correlation found between [GABA] in the sensorimotor area and PMd (Greenhouse *et al.*, 2016).

Taken together, these results may inform a potential change clinical guidance towards baclofen use: if baclofen administration impairs motor learning in healthy people, further studies should investigate whether it also impairs motor rehabilitation in patients who receive it as a muscle relaxant. In previous experiments studying older healthy people, motor learning was not associated with decreases in [GABA] but learning-related changes in GABA significantly correlated with motor learning improvements, age, and baseline GABA (King *et al.*, 2020). Pathological changes in [GABA] are associated with motor impairments in diseases such as stroke, multiple sclerosis or autism spectrum disorder and multiple sclerosis (Cawley *et al.*, 2015; Umesawa *et al.*, 2020; Grigoras & Stagg, 2021). Stroke survivors with a previous history of receiving GABA agonists had a significantly worse motor function on admission, but the administration of GABA agonists had no significant effect on motor rehabilitation outcomes (Johnstone *et al.*, 2021*a*). However, this retrospective study included both $GABA_A$ and $GABA_B$ receptors agonists in this analysis, which may differently affect motor learning. Baclofen is routinely administered to patients in multiple doses per day, which might have different effects to the single dose I administered in our study. Moreover, these agents are sometimes administered at night or when needed, so their action might not have overlapped with the rehabilitation window. The indications for which these drugs are given (spasticity, anxiety, insomnia) might affect the patients' lives and rehabilitation more if untreated. Considering the results of this study and others indicating impairments in motor learning after baclofen administration (Willerslev-Olsen *et al.*, 2011; Johnstone *et al.*, 2021*b*), the use of baclofen

in stroke should be reconsidered based on the severity of spasticity and post-stroke motor function and only administered if an alternative non-GABAergic muscle relaxant cannot be used.

3.4.3 The relationship between motor function and MRSI-derived [GABA]

3.4.3.1 Higher [GABA] in the premotor cortex, but not M1, is associated with slower response times

I found a significant correlation between response times to visual stimuli and [GABA] in the left premotor cortex during the placebo sessions, but not after baclofen administration. The premotor cortex is known to be involved in motor planning and sensorimotor integration (Riehle & Requin, 1989; Churchland *et al.*, 2006*c*; Kantak *et al.*, 2012; Shenoy *et al.*, 2013). fMRI studies investigating the brain areas active during simple response time tasks show a cortical network linked to the identification of the visual stimuli and movement execution, which includes the bilateral premotor cortices (Kansaku *et al.*, 2004). The premotor cortex has strong connections to the ipsilateral M1 (Muakkassa & Strick, 1979; Godschalk *et al.*, 1984; Camarda & Bonavita, 1985; Dum & Strick, 2005), but sends very sparse projections to the spinal cord (Dum & Strick, 1991). Further evidence of its involvement in movement production is that electrical intracortical pulses to the premotor cortex evoke simple movements in monkeys (Gentilucci *et al.*, 1988; Hepp-Reymond *et al.*, 1994). Moreover, delivering single TMS pulses to the premotor cortex at less than 25ms after the visual stimulus leads to faster RTs, suggesting that preconditioning the premotor cortex decreases the time needed for motor execution (Zangrandi *et al.*, 2019). Our results are in line with these studies, showing that lower inhibition in the premotor cortex is associated with faster response times.

The interaction between the premotor cortex and M1 has been probed using brain stimulation studies, which revealed that the effect of stimulating the premotor cortex on M1 depends on motor status, the intensity of the pulse and need for motor reprogramming. The premotor cortex seems to have an inhibitory effect on M1 during rest, with high-intensity conditioning pulses, or when motor reprogramming was necessary; however, it has a facilitatory effect during precision grip or with lower intensity conditioning pulses (Davare *et al.*, 2008; Bäumer *et al.*, 2009; Buch *et al.*, 2010). Micro-stimulation of the premotor cortex, but not motor cortex, during the preparatory activity, led to slower response time in monkeys. This is likely because the cortical area needs time to restore preparatory activity (Churchland & Shenoy, 2007*b*). Taken together, this evidence shows that the premotor cortex contributes significantly to motor planning and execution; individual variability in [GABA] may be the neural substrate for individual variability in response times.

There was no significant correlation between response times and left M1 [GABA] during either of the sessions. This is in contrast with previous studies showing that slower response times are associated with higher M1 [GABA] (Stagg *et al.*, 2011*a*) and that higher corticospinal excitability is associated with faster RTs (Sinclair & Hammond, 2008; Greenhouse *et al.*, 2017). However, the MRS voxel in Stagg 2011 was 8 cm^3 as opposed to our much smaller MRSI voxels of 0.375 cm^3. Although the MRS voxel was focused on M1, it did include regions of the premotor cortex, which might have been driving the correlation. Additionally, TMS pulses administered to M1 may have also affected the excitability of the premotor cortex, which could have contributed to the faster response times found there.

3.4.3.2 Larger [GABA] decreases in the premotor cortex during learning are associated with better motor learning in the baclofen session, but not placebo

I found a significant positive correlation between learning-related changes in the premotor cortex [GABA] and learning-related changes in RTs during SRTT during the baclofen session, but not placebo. Specifically, better learning was associated with a larger decrease in the premotor cortex [GABA] during motor learning. I did not find any significant correlation between learning-related changes in M1 [GABA] and learning-related changes in behavioural metrics in either of the two sessions.

Since the premotor cortex is involved in motor planning and movement preparation, it is not surprising that it has an important contribution towards motor learning (Kantak *et al.*, 2012), being linked to associative learning (sensory cues become associated with appropriate motor commands) in both healthy people and patients (Halsband & Freund, 1990; Nowak *et al.*, 2009; Steele & Penhune, 2010), as well as implicit motor sequence learning and imitation learning (Bischoff-Grethe *et al.*, 2004; Vogt *et al.*, 2007). Moreover, reorganisation of the premotor cortex was associated with improvements in motor function after stroke (Frost *et al.*, 2003; Fridman *et al.*, 2004).

Higher PMd activation during motor learning has also been associated with higher cognitive demands of the task (Cross *et al.*, 2007) and better behavioural outcomes at a motor-learning task (Tomassini *et al.*, 2011). Therefore, it is possible that during the baclofen session, when GABAergic inhibition is higher in M1, the premotor cortex

contributes more to the process of motor learning and the individual variability in motor learning reflects the variability of learning-related [GABA] decreases.

I did not find any correlation between learning and [GABA] change in M1. Both M1 and premotor cortex have been reported as the location for the engram after SRTT performance (Hamano *et al.*, 2020) and larger NIBS-induced GABA decreases in M1 are correlated with better motor learning (Stagg *et al.*, 2011*a*). The change in the GABA/Glu ratio is also reported to be negatively correlated with behavioural change during SRTT (Maruyama *et al.*, 2021), although the common synthesis pathway for GABA and glutamate makes this finding difficult to interpret. I collected the MRSI data for 9 minutes after the end of the SRTT task. It is possible that MRSI measurements collected after task competition might not be directly reflective of the GABA dynamics during the task, if [GABA] quickly returns to pre-task values once the motor performance ends.

3.5 CONCLUSION

I conducted a within-subject, double-blind, placebo-controlled study to investigate how increasing GABAergic inhibition via pharmacological agent baclofen affected motor learning and brain chemistry. I found that baclofen significantly impaired performance on a motor sequence learning task, but did not impair simple response times or action-selection times. These behavioural impairments were associated with significant learning-related changes in [GABA] in the left M1 and left premotor cortex. These results are of clinical importance: baclofen is often administered to stroke survivors as a muscle relaxant, and it might impair their motor rehabilitation.

CHAPTER 4 - THE EFFECT OF BACLOFEN ON PATTERNS OF BRAIN ACTIVITY DURING TASK PERFORMANCE

In the previous chapter, I explored the effects of modulating GABAergic inhibition on motor learning and brain chemistry. I found that pharmacologically increasing GABAergic inhibition using baclofen led to impaired performance on a motor sequence learning task and learning-related changes in [GABA] dynamics. Motor behaviour relies on the correct activation of the motor network, which includes M1, premotor cortex, supplementary motor areas and subcortical structures, and different types of motor learning are associated with distinct patterns of activation within this network (Hardwick et al., 2013). Here, I investigated how increasing GABAergic inhibition affected the patterns of brain activation during motor sequence learning. I hypothesised that baclofen would decrease the activity of the motor sequence learning network and that the baclofen-related impairments in motor sequence learning would be associated with additional brain areas being active during motor learning, as an adaptive mechanism.

4.1 INTRODUCTION

4.1.1 Structural and functional connectivity

Distinct brain regions do not function independently; instead, they are connected to other regions through short- and long-range connections (Park & Friston, 2013). For example, while the cortical projections onto the motoneurons in the spinal cord come from the primary motor cortex, M1 is not the only area active during motor behaviour. The motor network encompasses numerous brain regions that contribute to motor control, including

bilateral M1s, adjacent areas (the premotor cortex, supplementary motor area) and subcortical structures (basal ganglia, thalamus and cerebellum).

Both short- and long-range connections characterise structural connectivity: brain areas have input/output projections from/to adjacent areas that contribute to similar processes, especially seen in motor and sensory systems; more distal projections are thought to be involved in more complex cognitive processes (Bullmore & Sporns, 2009; Sepulcre *et al.*, 2010). Functional connectivity, however, does not necessarily require an organic connection between two brain regions: the activity of two brain regions can fluctuate synchronously if they are structurally connected, but also if they both receive projections from the same third area (Adachi *et al.*, 2012; Park & Friston, 2013). Structural and functional connectivity are closely related: functionally related areas are more likely to be structurally connected and reconfiguration of functional connectivity depends on the underlying structural connections (Honey *et al.*, 2009). The physiological mechanisms of functional connectivity are not yet fully understood, but synchronised activity in different brain regions is thought to be mediated by brain oscillations (Salinas & Sejnowski, 2001; Fries, 2005); techniques measuring oscillatory activity, such as magnetoencephalography, reveal resting-state networks similar to fMRI-derived ones (Brookes *et al.*, 2011). Brain oscillations can affect neurovascular coupling, which is how we can measure synchronised activity in networks using fMRI: fluctuations in gamma-band amplitude entrain vasomotor oscillations in pial arterioles (Mateo *et al.*, 2017; Drew *et al.*, 2020).

4.1.2 Task-based networks

Task fMRI has been used extensively in neuroscience research to study patterns of brain activity during task performance. Analysis of task fMRI data led to the identification of distinct task-based networks, e.g. action-selection network, motor learning network, attention network or face-processing network (Posner, 2012; Hardwick *et al.*, 2013; O'Shea *et al.*, 2014; Müller *et al.*, 2018) that are dependent on the question being investigated and the task used.

Of particular interest here are the networks that are active during the performance of motor tasks: motor execution, action-selection and motor learning. The brain areas that are active during motor execution are the contralateral primary motor cortex, premotor cortex and supplementary motor area (SMA) and cerebellum (Hanakawa *et al.*, 2008). The action-selection network is a bilateral premotor-parietal network, which is active when selecting the correct motor response to a stimulus (O'Shea *et al.*, 2014). The "core" motor learning network includes the primary motor cortex, dorsal premotor cortex, SMA, primary somatosensory cortex, superior parietal lobule, thalamus, putamen and cerebellum, although changes in the patterns of activation for these areas might appear depending on the type of motor learning task used (Hardwick *et al.*, 2013). Specifically, motor sequence learning tasks are associated with higher activity in the cortical areas and the thalamus than sensorimotor tasks, which in turn are associated with higher activity in the basal ganglia and the cerebellum (Hardwick *et al.*, 2013).

Baclofen has been used extensively in fMRI studies investigating addictive behaviour (Young *et al.*, 2014; Beck *et al.*, 2018; Logge *et al.*, 2021; Morley *et al.*, 2021), but its effect on task-based networks in healthy people is still unclear.

4.1.3 Aim

I conducted a within-subject, double-blind, placebo-controlled study to investigate the effect of a single dose of baclofen (20mg) on patterns of brain activity during motor learning and at rest. Since motor learning is associated with decreases in M1 [GABA], **I hypothesised that modulating GABAergic inhibition would decrease the activity of the motor sequence learning network and additional brain areas would be recruited to support task performance.**

4.2 METHODS

4.2.1 Experimental design

As previously described in Chapter 3, I recruited 18 young, healthy volunteers to take part in this study. All volunteers participated in two sessions at least one week apart; the sessions were identical except on one they received a dose of 20mg of baclofen and, on the other, they received a placebo pill (sucrose/lactose). During each session, participants had an MRI scan, which included a resting-state fMRI scan and two task fMRI scans: one during the action-selection task performance and one during serial reaction time task performance; both tasks were described in more detail in Chapter 3. Three participants could not complete the MRI scans due to claustrophobia, so all further analyses only included data from 15 participants.

4.2.2 MRI Data Acquisition

All MRI data were acquired in a 3T Siemens Magneton System (PRISMA) using a 32-channel head coil (Nova Medical Inc, Wilmington, USA), as described in Chapter 3. Foam wedges were used as padding to minimise head motion. Using an angled mirror, participants could see stimuli on a screen behind them. First, a T1-weighted structural scan was acquired (Magnetisation Prepared Rapid Acquisition Gradient Echo sequence, TR = 1.9s, TE = 3.96ms, TA = 7.3min, 1mm isotropic). This was followed by a resting-state fMRI scan (matched to the resting-state UK Biobank parameters (Alfaro-Almagro *et al.*, 2018): T2*-weighted echo-planar imaging (EPI) sequence, TR = 0.735s, TE = 39ms, slice thickness = 2.40mm, TA = 6.16min), where participants were asked to stay awake, keep their eyes open and look at a fixation cross on the screen. Then, the pre-task MRSI scan was acquired (semi-LASER DW-CRT sequence with metabolite cycling (Steel *et al.*, 2018),

TR = 1.4s, TE = 32ms, TA = 2 x 4.5min, voxel size = 5 x 5 x 15 mm^3). Two task fMRI scans were then acquired using a T2*-weighted EPI sequence (TR = 0.933ms, TE = 33.40ms, 2mm isotropic), one for the action-selection task (TA = 6.3min) and one for the SRTT (TA = 15.3min), the motor tasks having been described in Chapter 3. Lastly, a post-task MRSI scan was acquired (as above). MRSI data acquisition, processing and analysis were described in more detail in Chapter 3.

After acquisition, all task fMRI data were organised in accordance with the Brain Imaging Data Structure (Gorgolewski *et al.*, 2016).

4.2.3 Task fMRI analysis

4.2.3.1 Lower-level analysis

Task fMRI analysis was conducted using the FMRIB Software Library (FSL, Oxford) (Jenkinson *et al.*, 2012*a*). Pre-processing for the single-session analysis included motion correction using Motion Correction FMRIB's Linear Image Registration Tool (MCFLIRT; (Jenkinson *et al.*, 2002*a*)), brain extraction and bias-field correction using fsl_anat, which included the FSL Brain Extraction Tool and FMRIB's Automated Segmentation Tool (Zhang *et al.*, 2001; Smith, 2002), high-pass temporal filtering (90s), spatial smoothing with a 5mm FWHM Gaussian kernel and B0 fieldmap unwarping. Each participant's fMRI data was registered to their structural scan using linear (FMRIB's Linear Image Registration Tool; FLIRT) and non-linear (FMRIB's Non-linear Image Registration Tool; FNIRT) registration (Jenkinson & Smith, 2001; Jenkinson *et al.*, 2002*a*; Smith *et al.*, 2004).

For the action-selection task, the fMRI data was modelled using a general linear model (GLM) with 2 explanatory variables (EVs), one for execute blocks and one for select blocks.

These two EVs were convolved using double-gamma haemodynamic response function and head motion parameters were added as confounds. Brain activity during AS task performance was investigated using five contrasts: mean (both types of blocks), execute blocks only, select blocks only, execute > select and select > execute.

For the SRTT, the fMRI was modelled using a GLM with 15 EVs, one for each task block. All EVs were convolved using double-gamma haemodynamic response function and head motion parameters were added as confounds. Brain activity during SRTT task performance was investigated using multiple contrasts: mean (across all blocks), random (the 2 random blocks), learning (sequence blocks 1 to 11 (S1 to S11)), early > late learning (S1 > (S10 to S13)), early < late learning (S1 < (S10 to S13)), random > learning ((R1 and R2) > (S1 to S11)) and random < learning (((R1 and R2) < (S1 to S11)).

4.2.3.2 Higher-level analysis

For each motor task, higher-level (group-level) analysis was conducted using a paired t-test design investigating the effect of the treatment, and FSL Local Analysis of Mixed Effects (FLAME 1+2 (Beckmann *et al.*, 2003)). In order to study the effect of baclofen on the task network, I applied the task mean activation map (across both sessions and all blocks for each task) as a pre-threshold map for the respective higher-level analysis. To study whether baclofen recruited additional brain areas for task performance, I also performed the higher-level analysis without the pre-threshold mask. Significant voxel clusters were identified using randomise, FSL's tool for nonparametric permutation inference, with Threshold-Free Cluster Enhancement and cluster-based threshold outputs for Z values over 3.1 and p values < 0.05 FEW-corrected (Winkler *et al.*, 2014). Brain areas were identified using the Harvard-Oxford Cortical Structural Atlas (Makris *et al.*, 2006;

Desikan *et al.*, 2006; Goldstein *et al.*, 2007). For specific contrasts, I calculated the mean values for the contrast of parameter estimates (COPE) values within the significant voxel clusters using fslmeants (FSLUTILS, Oxford).

For the AS task, I found similar group-level results when conducting the analysis with or without the execute response times of each participant as an additional EV. I also found similar SRTT group-level results when conducting the analysis with or without either the response times on random blocks or the learning gradients (calculated as described in Chapter 3) as additional EVs.

4.2.4 Working memory tests

Participants completed working memory tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) approximately 3 hours after treatment administration (Robbins *et al.*, 1994). Similar to previous studies, the following four tests were used to assess cognitive functions, including working memory and alertness: Spatial Working Memory (SWM), Pattern Recognition Memory (PRM), Spatial Span (SSP), and Rapid Visual Processing (RVP). Due to technical difficulties, 3 participants could not complete the SWM, PRM and RVP tests on one of the sessions.

4.2.4.1 Spatial working memory

This test was sensitive to the participants' visuospatial skills. A number of boxes appeared on the screen and participants were instructed to tap one box to find out whether there was a token in it. Only one token appeared in each of the boxes, so participants had to remember which boxes they had already found tokens in and which boxes they still needed to check. Participants committed errors whenever they tapped a box in which

they had already looked for a second time: either it had a token in it, so it could not have one again, or it did not have a token in it, so they should have checked a different box. The number of total errors was reported for increasing task difficulties (4, 6, 8 and 12 blocks). A strategy metric was also recorded, quantifying how many times the participants had started searching from the same block, indicating a strategy for task performance.

4.2.4.2 Pattern Recognition Memory

This test probed skills in recognising visual patterns. Participants were presented with a set of shapes, which they were instructed to remember. Pattern recognition memory was evaluated at two time points: immediately after the presentation of the set of shapes and at approximately 20 minutes after it. Participants were asked to recall the shapes they remembered from 2 possible options. The percentage of correct choices and the response times for pattern recognition were recorded for both the immediate and delayed time points.

4.2.4.3 Rapid Visual Processing

This test interrogated the participants' attention. Digits between 2 and 9 were briefly displayed in the centre of the screen in a pseudorandom order. Participants were instructed to tap a red button on the screen if the following target sequences appeared: 3-5-7, 2-4-6 and 4-6-8. I recorded three metrics: the accuracy of sequence detection, median response times and the probability of false alarm.

4.2.4.4 Spatial Span

This test evaluated the participants' working memory. For each trial, a set of 9 boxes would appear on the screen, out of which a subset would change colour one at a time, forming a sequence. In the forward version of this task, participants were asked to tap the boxes in the order they had lit up previously; in the reverse version, participants were asked to tap the boxes in the reserve order. The sequence gradually increased from 2 to 9 boxes, making the task more difficult to perform. The length of the most difficult correctly-recalled sequence was recorded, as well as the number of errors made, for both the forward and the reverse spatial span.

4.2.5 Mood questionnaires

To quantify any baclofen-related changes in mood, I asked participants to fill in the Bond-Lader Visual Analogue scale (BLVAS) three times during each session: before treatment administration (baseline), just before the MRI scan at 1 hour after treatment administration (1h) and immediately after the MRI scan (2h). The BLVAS has 16 scales, each 100mm long with the 2 opposite sides of the spectrum at each end, e.g. alert – drowsy, calm – excited (Bond & Lader, 1974). At each time point, participants were instructed to make a mark on each scale for how they felt at that specific moment. Similar to previous studies, the 16 scales were grouped into 3 categories: alertness (alert, strong, clear-headed, well-coordinated, energetic, quick-witted, attentive, proficient, interested), contentedness (contented, tranquil, happy, social, friendly) and calmness (calm, relaxed) (Bond & Lader, 1974). The mean value for each category was calculated at each time point, lower values indicating stronger feelings of that respective scale.

4.2.6 Statistical analysis

Analysis of working memory tests and mood was performed using Prism (v.9.3.0, GraphPad Prism Software). Analysis was performed using repeated-measures ANOVA or paired t-tests with significance levels of α=0.05 to investigate the within-subject effects of baclofen compared to placebo. All graphs show mean+/-SD or individual data points unless otherwise indicated.

4.3 RESULTS

4.3.1 Mean activation maps revealed the brain areas involved in motor task performance

First, I investigated which brain areas are active during motor task performance (Fig. 4.1). As expected, areas involved in motor control were active for both action-selection and SRTT, including the primary motor and somatosensory cortex, premotor cortex, supplementary motor area, cerebellum, basal ganglia, and thalamus. SRTT task performance, but not action-selection, was associated with the activation of both frontal poles and the right superior parietal lobule.

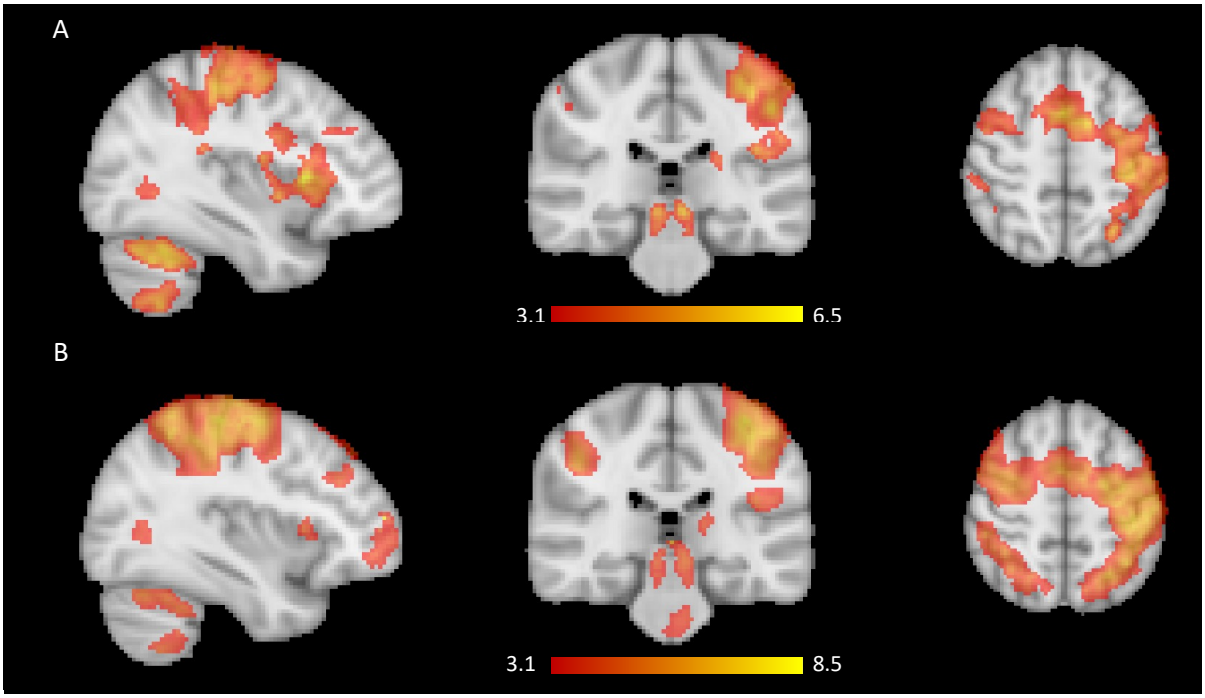


Fig. 4.1. **Mean activation maps for action-selection (A) and SRTT (B) task performance**. These maps were then used as pre-threshold masks in task group-level analysis

4.3.2 Baclofen did not significantly affect brain activity during action-selection task performance

I previously showed that baclofen did not significantly affect execute response times or action-selection times (Fig. 3.6). Here, I investigated whether baclofen affected the patterns of brain activity implicated in performing the action-selection task, by comparing brain activity across execute and select blocks between treatment conditions, both with and without the AS mean activation map as a pre-threshold mask. I found no voxels showing significantly different activity between baclofen and placebo conditions across all blocks of the task or across execute blocks alone, with or without the mask. This indicates that baclofen did not significantly change the activity within the action-selection network, and it was also not associated with the recruitment of additional brain areas during task performance.

4.3.3 Baclofen was associated with increased activity in frontal regions during SRTT task performance

I then investigated how baclofen affected the pattern of brain activity during SRTT task performance. Across all blocks, I found higher activity in the left frontal pole after baclofen administration compared to placebo (baclofen > placebo) (

Fig. 4.2A). I found similar results when studying only sequence blocks: higher activity in both frontal poles and medial prefrontal gyrus for baclofen > placebo (

Fig. 4.2B). After applying the SRTT mean activation map as a pre-threshold mask, I found no voxels with significantly different activity between treatment conditions, indicating

that these frontal cortex areas were not part of the network involved in task performance and were recruited after baclofen administration.

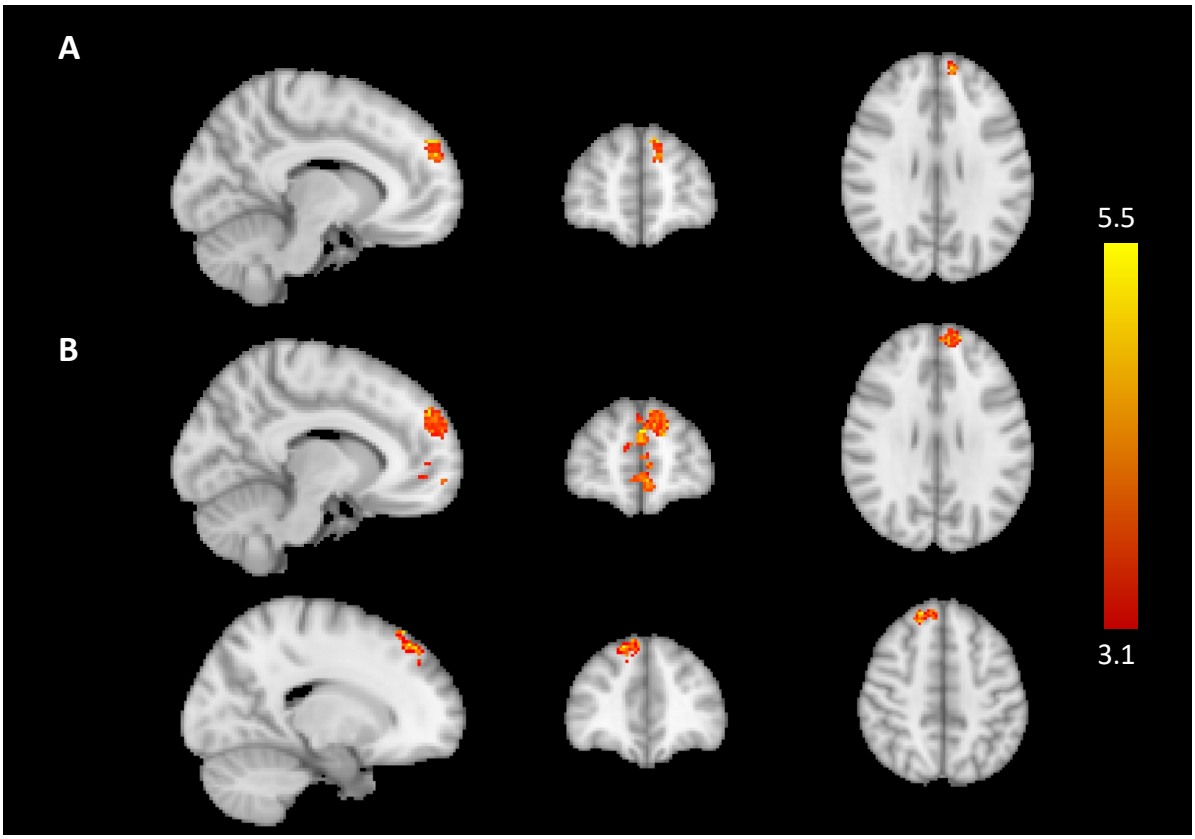


Fig. 4.2. **Voxels with significantly higher activity during SRTT blocks after baclofen administration compared to placebo.** (A) Higher activity in the left frontal pole across all SRTT blocks (random and sequence) with baclofen (i.e. baclofen – placebo); (B) Higher activity in frontal poles (left frontal pole – top; right frontal pole – bottom), paracingulate gyrus and frontal medial cortex during sequence blocks only with baclofen (i.e. baclofen – placebo)

In order to investigate the relationship between this activation in the frontal poles and behaviour, I performed a correlation analysis between the mean activation during sequence blocks in the areas in Fig.4.2B and the skill acquired during motor sequence learning (R2- S11). I found no significant correlation between activity in these areas of the frontal poles and skill (baclofen: $r(12) = 0.129$, $p = 0.660$; placebo: $r(12) = -0.077$, $p = 0.794$).

4.3.4 Baclofen affected brain activation patterns between early and late learning

I then investigated whether baclofen affected the brain activity changes during motor learning. I compared brain activity during the first sequence block and the last 4 sequence blocks (sequence blocks 10 to 13) between baclofen and placebo conditions. I found significant voxel clusters in the precuneus, anterior cingulate gyrus and paracingulate gyrus (baclofen (S1 > S10:S13) > placebo (S1 > S10:S13); Fig. 4.3A); the difference in these areas' activity between early and late sequence blocks was higher with baclofen, due to higher activity in the first sequence blocks and lower activity in the last 4 blocks (Fig. 4.3B). After applying the SRTT mean activation map as a pre-threshold mask, I found no significant voxel clusters, suggesting that these areas were not part of the group SRTT network, and that they might be recruited in early learning with baclofen.

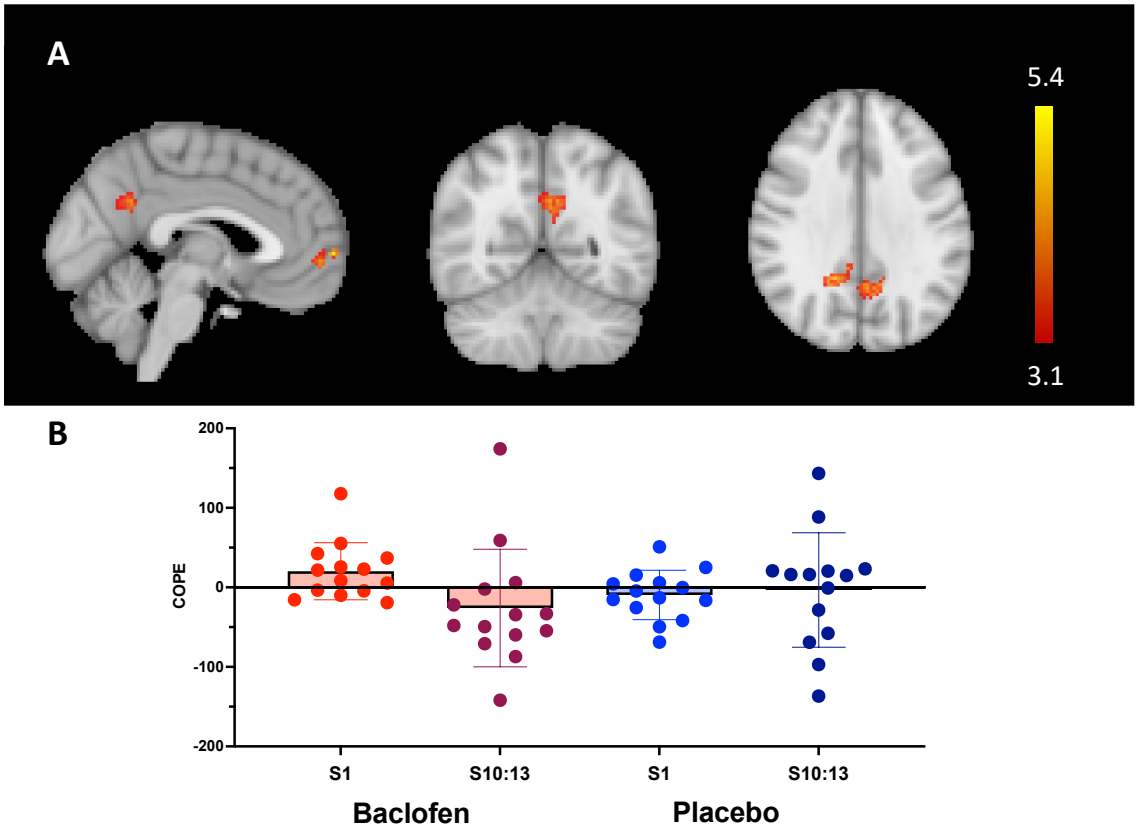


Fig. 4.3. **Areas of the precuneus, cingulate gyrus and paracingulate gyrus showed different patterns of activity during early and late motor sequence learning between treatment conditions** (A; baclofen (S1 > S10:S13) > placebo (S1 > S10:S13))); COPE values for first sequence blocks (S1) and sequence blocks 10 to 13 (S10:13) (B)

4.3.5 Baclofen was associated with decreased activity in the thalamus and basal ganglia during random blocks

I found lower activity in the left putamen and the left and right thalamus during random button presses after baclofen administration compared to placebo (baclofen (R1 & R2) < placebo (R1 & R2); Fig. 4.4A). I found similar results after applying the SRTT mean activation map as a pre-threshold mask: lower activity in the left putamen and thalamus with baclofen. I also found lower activity in the left primary motor cortex, left premotor cortex and right cerebellum during baclofen compared with placebo (baclofen (R1 & R2) < placebo (R1 & R2); Fig. 4.4B). This indicates that baclofen affected the within-network activity of the brain areas involved in the execution of random button presses.

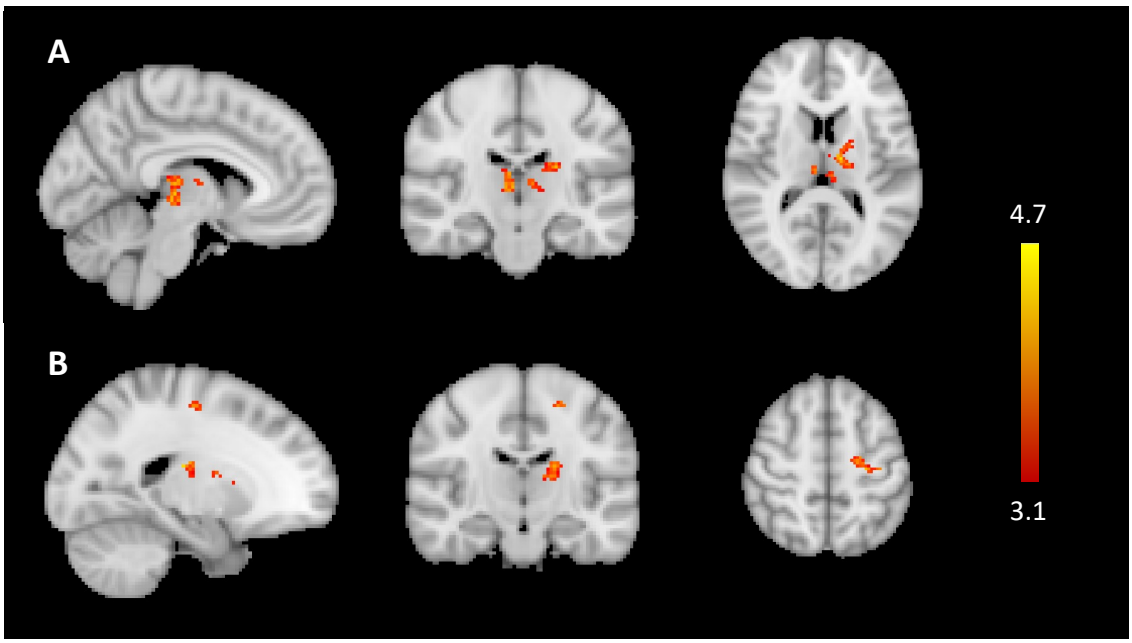


Fig. 4.4. **Subcortical areas showed decreased activity during random blocks after baclofen administration compared to placebo** (A; baclofen (R1 & R2) < placebo (R1 & R2)); some cortical and cerebellar areas also showed decreased activity with baclofen after the application of the SRTT mean activation map as a pre-threshold mask (B)

4.3.6 Baclofen affected the changes in brain activity patterns between random and sequence blocks

I then investigated how baclofen impacted brain activity patterns between random and sequence blocks, by comparing the difference in brain activity during the 2 random blocks and the 11 sequence blocks between treatment conditions. There were significant clusters in the angular gyrus, left M1, middle temporal gyrus, frontal orbital cortex and frontal pole (baclofen (sequence > random) > placebo (sequence > random); Fig. 4.5A). These areas have lower activity during random blocks and higher activity during sequence blocks in the baclofen session compared to placebo (Fig. 4.5B). After applying the SRTT mean activation map as a pre-thresholding mask, there was only a significant cluster in the supplementary motor area, with lower activation during random blocks with baclofen (baclofen (sequence > random) > placebo (sequence > random); Fig. 4.5C-D). Therefore, baclofen was associated with decreased activity in an area of the SRTT network and recruitment of additional brain regions during motor sequence learning.

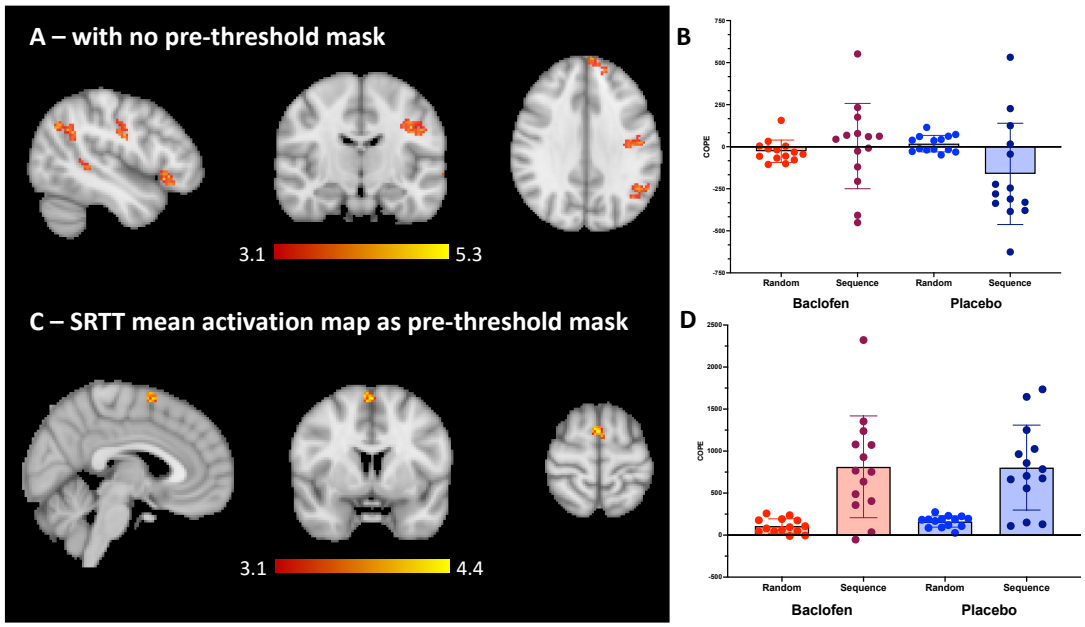


Fig. 4.5. **Areas of increased learning-related activity (sequence > random) with baclofen** (baclofen (sequence > random) > placebo (sequence > random)), without a pre-threshold mask (A) and with the SRTT mean activation map as a pre-threshold mask (C). Corresponding COPE values in (B) and (D)

4.3.7 Baclofen did not significantly alter performance on working memory tests

I next investigated whether baclofen affected working memory on a battery of CANTAB computerised working memory tests.

4.3.7.1 Spatial working memory

First, I focused on the participants' performance on the spatial working memory task (Fig. 4.6). I compared the number of errors made during the task between treatment conditions using a two-way repeated-measures ANOVA with within-subject treatment factors (baclofen, placebo) and task difficulty (4, 6, 8 and 12 blocks). I found a significant effect of difficulty ($F(1.084, 18.43) = 65.66$, $p < 0.0001$), but no significant effect of treatment ($F(1, 17) = 0.335$, $p = 0.5704$) and no significant treatment x difficulty interaction ($F(1.181, 17.71) = 0.214$, $p = 0.689$; Fig. 4.6A left). I also compared the strategy score for the task between treatment conditions and found no significant results (paired t-test: $t(14) = 0.1094$, $p = 0.914$; Fig. 4.6A right). These results suggest that baclofen did not significantly impair spatial working memory.

4.3.7.2 Pattern recognition memory

I then looked at the participants' performance on a pattern recognition memory task (Fig. 4.6B). Using two-way repeated-measures ANOVAs with within-subject factors of treatment (baclofen, placebo) and latency (immediate, delayed), I found no significant effect of treatment on either accuracy (treatment $F(1, 17) = 1.294$, $p = 0.271$; latency $F(1, 17) = 2.766$, $p = 0.1146$; treatment x latency interaction $F(1,15) = 0.629$, $p = 0.440$; Fig. 4.6B left) or response times (treatment $F(1, 17) = 1.792$, $p = 0.198$; latency $F(1, 17) = 6.731$,

p = 0.0.019; treatment x latency interaction *F(1,15)* = 0.503, *p* = 0.489; Fig. 4.6B right). Therefore, baclofen did not significantly affect pattern recognition.

4.3.7.3 Rapid visual processing

In order to evaluate the effect of baclofen on attention, I compared participants performance on a rapid visual processing task between treatment conditions (Fig. 4.6C). I found no significant effect of treatment on either performance scores (paired t-test *t(15)* = 0.172, *p* = 0.866; Fig. 4.6C left), response times (paired t-test *t(15)* = 1.759, *p* = 0.099; Fig. 4.6C middle) or probability of a false alarm (paired t-test, *t(15)* = 1.218, *p* = 0.242; Fig. 4.6C right), indicating that baclofen did not significantly affect attention.

4.3.7.4 Spatial span

I then used the spatial span task to evaluate the participants' working memory in a task similar to the digit span but using spatial cues instead of digits (Fig. 4.6D). Using two-way repeated-measures ANOVAs with within-subject factors of treatment (baclofen, placebo) and difficulty (forward, reverse), I found no significant effect of treatment on either span length (treatment *F(1, 17)* = 2.227, *p* = 0.154; difficulty *F(1, 17)* = 0.979, *p* = 0.336; treatment x difficulty interaction *F(1,17)* = 0.308, *p* = 0.586; Fig. 4.6D left) or number of errors (treatment *F(1, 17)* = 0.221, *p* = 0.644; difficulty *F(1, 17)* = 0.086, *p* = 0.773; treatment x difficulty interaction *p* = 0.772, *F(1,17)* = 0.0.087; Fig. 4.6D right).

Taken together, these results indicate that baclofen did not significantly impair working memory or attention.

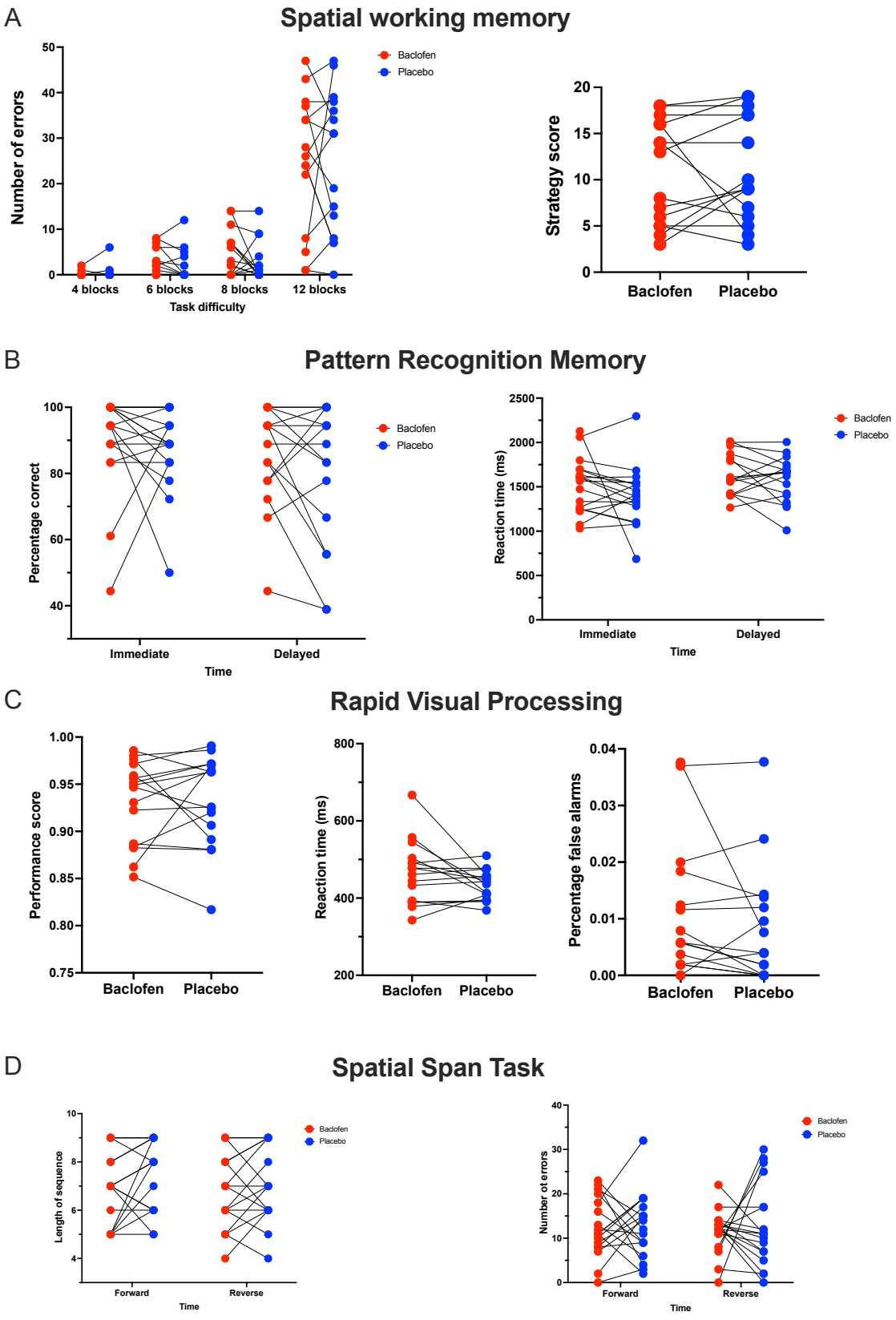


Fig. 4.6. **No significant effect of baclofen on a CANTAB computerised battery of working memory tests**

4.3.8 Baclofen significantly affected contentedness, but not alertness or calm

Lastly, I interrogated the effect of baclofen on the participants' mood. As previously described, the 16 BLVAS scales were loaded onto three mood components: alertness (alert, strong, clear-headed, well-coordinated, energetic, quick-witted, attentive, proficient and interested), contentedness (contented, tranquil, happy, sociable and friendly) and calmness (calm and relaxed). The mean value for each mood component was measured on each session at 3 time points: before treatment administration (baseline), 1h and 2.5 hours after treatment administration. To determine the effect of baclofen on each mood component, I used a two-way repeated-measures ANOVAs with within-subject factors of treatment (baclofen, placebo) and time (baseline, 1h, 2.5h).

I found a significant effect of time on alertness, but no significant effect of treatment or treatment x time interaction (treatment, $F(1, 14) = 4.164$, $p = 0.061$; time $F(1.775, 24.86) = 11.18$, $p = 0.0005$; treatment x time interaction, $F(1.348, 18.88) = 2.389$, $p = 0.132$; Fig. 4.7A).

I found a significant effect of treatment on contentedness, but no significant effect of time or treatment x time interaction (treatment $F(1, 14) = 5.142$, $p = 0.038$; time $F(1.362,19.07) = 0.385$, $p = 0.607$; treatment x time interaction $F(1.945, 27.23) = 0.899$, $p = 0.416$; Fig. 4.7B).

I found no significant effects of time or treatment on calmness (treatment $F(1, 14) = 0.653$, $p = 0.433$; time $F(1.556, 21.78) = 0.385$, $p = 0.797$; treatment x time interaction $F(1.310, 18.34) = 0.163$, $p = 0.425$; Fig. 4.7C).

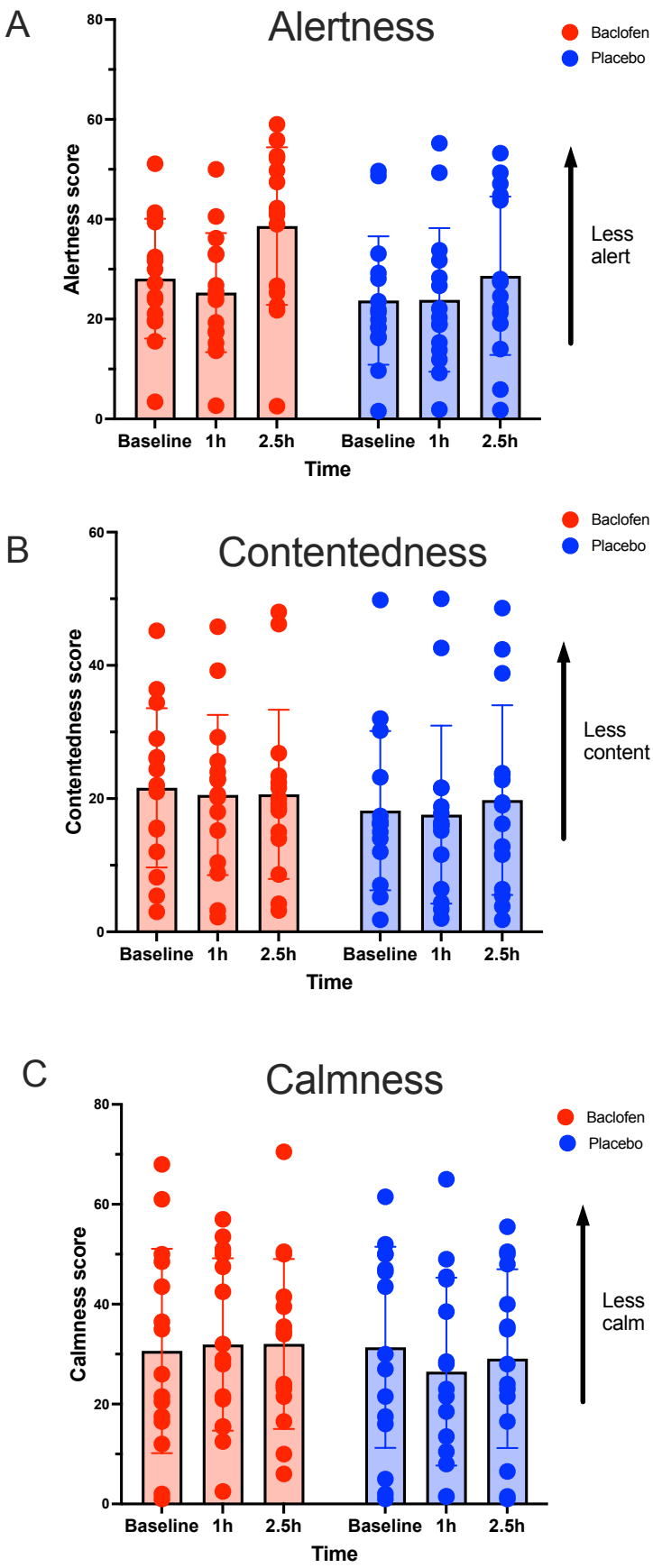


Fig. 4.7. **The effect of baclofen on mood components**: alertness (A), contentedness (B) and calmness (C). Columns and bars represent mean +/- SD.

4.4 Discussion

Here, I studied the effect of baclofen on task-based (AS, SRTT) networks during motor execution and motor sequence learning to investigate the role o GABAergic inhibition in healthy motor learning. While I found no baclofen-related changes in the AS network, I found significant baclofen-related changes in the patterns of brain activity during SRTT. Noteworthy, baclofen was associated with increased activity in the frontal pole during sequence learning blocks and decreased activity in subcortical structures during random blocks. Lastly, I assessed whether baclofen had a significant effect on other cognitive domains, and I found no effect of baclofen on working memory or alertness.

4.4.1 Baclofen did not significantly affect brain activation patterns during the action-selection task performance

I found no significant activity changes in the action-selection network between treatment conditions. This was expected, as I also did not find any behavioural effects of baclofen on either response times in response to a visual stimulus or action-selection times in both this study (Chapter 3, Fig. 3.6) and a previous pharmaco-TMS study conducted in the lab (Johnstone, 2019). Baclofen treatment can improve voluntary motor function in patients with spasticity (Latash *et al.*, 1989) and can inhibit motor execution in rats (Paredes & Agmo, 1989), but the strength of the antispastic effect of baclofen and its dose-dependency in healthy people has not been investigated. It is possible that either the antispastic effect of baclofen is not strong enough in healthy people or our dose (20mg) was too small to elicit a behavioural effect on a simple motor task. It is also possible that compensatory spinal mechanisms are engaged to maintain normal motor execution. While a GABA-mediated improvement in action-selection has been reported in stop-

change and Go/No-Go tasks (Steenbergen *et al.*, 2015; Quetscher *et al.*, 2015), I did not find such an effect in our action-selection task. The action-selection network might rely more on $GABA_A$-mediated mechanisms than $GABA_B$, which would be why I did not see any significant changes in action-selection with baclofen. Moreover, the Go/No-go network is known to rely on response inhibition mechanisms mediated by the basal ganglia (Jahanshahi *et al.*, 2015), which is different to the bilateral premotor-parietal action-selection network I identified here.

4.4.2 Baclofen was associated with significant changes in brain activity patterns during SRTT performance

4.4.2.1 Increased activity in frontal regions during SRTT sequence blocks

Baclofen was associated with increased activity in the frontal pole and medial prefrontal gyrus across all SRTT sequence blocks. These areas were not part of the mean SRTT activation map, indicating that they were recruited during task performance in the baclofen, but not placebo, session.

The frontal pole is an evolutionary new brain area, thought to be unique to higher primates (Preuss & Goldman-Rakic, 1991; Semendeferi *et al.*, 2001; Tsujimoto *et al.*, 2011). Its role is not completely understood, having been implicated in multitasking, episodic memory, attention, mentalising and integrating cognitive processes (Gilbert *et al.*, 2006; Tsujimoto *et al.*, 2011). Of relevance to motor behaviour, significant activation of the frontal poles has been reported during the execution of spontaneous voluntary movements (Hunter *et al.*, 2004). The frontal pole has also been implicated in sequence processing, especially of auditory stimuli, and prospective memory (Okuda *et al.*, 1998; Wang *et al.*, 2015; Wilson *et al.*, 2015, 2017). Meanwhile, the medial prefrontal cortex

contributes to error prediction and cognitive flexibility (Koechlin *et al.*, 2002; Kim *et al.*, 2011; Zarr & Brown, 2016) and plays a role in episodic memory by integrating information over multiple cognitive domains (Bowman & Zeithamova, 2018). Both the frontal pole and medial prefrontal cortex contribute to goal-directed behaviour, by encoding the value of the adapting behaviour and the current decision, respectively (Boorman *et al.*, 2009; Hosoda *et al.*, 2020).

Considering their role in sequence processing and memory, it is perhaps surprising that these areas are not included in the mean SRTT activation map; the reason for this might be that our SRTT task is not highly abstract. Different levels of abstraction are represented in different areas of the frontal lobe, with more rostral areas being activated by more abstract concepts and rules (Badre & D'Esposito, 2007; Nee & Brown, 2012; Zarr & Brown, 2016) and increased cognitive control (Bahlmann *et al.*, 2012). Therefore, it is likely that while the activity of these regions is not necessary for task performance during the placebo session, they are recruited as an adaptive mechanism for the increased GABAergic inhibition in the SRTT network. It is also possible these regions are active during the baclofen session due to more cognitive control being necessary for task performance after increased GABAergic inhibition. Indeed, the frontal pole plays a role in integrating cognitive processes, as it is connected to the default mode network and the executive control network (Moayedi *et al.*, 2015).

4.4.2.2 Different patterns of activity between early and late learning

I then investigated whether the changes in brain activity patterns from early to late learning were different between baclofen and placebo. I found that voxel clusters in the precuneus, cingulate and paracingulate gyrus showed a higher learning-related change (early > late) in the baclofen session compared to placebo. These areas are not part of the mean SRTT activation map, as no significant voxels were found for this contrast when applying the mean activation map as a pre-threshold mask.

The precuneus is the posteromedial segment of the parietal lobe and has rich bidirectional connections with other parietal areas, as well as the prefrontal cortex, premotor and supplementary motor area (Cavanna & Trimble, 2006). Neuroimaging studies investigating the role of the precuneus report that is it implicated in the coordination of bimanual motor behaviour, attention, motor and memory-related imagery, and self-processing, which is not surprising considering its strong connectivity with sensorimotor and associative brain areas (Nagahama *et al.*, 1999; Kircher *et al.*, 2002; Simon *et al.*, 2002; Hanakawa *et al.*, 2003; Wenderoth *et al.*, 2005; Cavanna & Trimble, 2006, 2006). The anterior cingulate and paracingulate cortex are a part of the medial prefrontal cortex and play an important role in attentional processes (Shenhav *et al.*, 2013; Wei *et al.*, 2013; Gennari *et al.*, 2018). There is high individual variability in the morphometry of these regions which affects cognitive functions, with leftward asymmetry of the paracingulate gyrus being associated with better performance on executive tasks (Yücel *et al.*, 2001; Fornito *et al.*, 2004, 2008).

Together, these regions are part of a parietal memory network, where novel stimuli are associated with decreases in network activity and familiar (pre-learnt) stimuli are

associated with increases in the network activity (Gilmore *et al.*, 2015). This pattern of activity in the association cortex during learning has also been reported in rodents (Gilad & Helmchen, 2020). I found that baclofen affected this pattern of brain activity during motor learning, which was associated with behavioural impairments.

These regions are also part of the default mode network, a network with higher activity at rest, which decreases during task performance (Utevsky *et al.*, 2014; Raichle, 2015). It is possible that the attentional processes mediated by these regions were affected by the increased GABAergic inhibition induced by baclofen: compared to the placebo session, these already inhibited DMN regions showed reduced activity change, which may have contributed to the behavioural impairment. In support of this hypothesis, the connectivity between the precuneus and other cortical regions (dorsal prefrontal and visual cortices) increases after the administration of anaesthetic agents, like propofol (Liu *et al.*, 2014), which increase GABA-mediated inhibition.

4.4.2.3 Decreased activity in subcortical structures during random blocks

I then investigated how baclofen affected patterns of brain activity when participants were pressing buttons in a random order (no learning). I found significantly lower activity in subcortical areas (left putamen and bilateral thalamus) in the baclofen session compared to placebo. Similar results were present after applying the SRTT mean activation map as a pre-threshold mask, with the addition of lower activity in cortical structures (left primary motor cortex, left premotor cortex and right cerebellum). This shows that baclofen affected brain activity during the execution of random button

presses, despite there being no behavioural differences in response times for random button presses between treatment conditions.

Both the putamen and the thalamus are part of the cortico-basal ganglia-thalamocortical circuit and, as such, are heavily connected between themselves and with cortical motor regions (primary motor cortex, premotor cortex, supplementary motor areas). Both regions have long been known to play an important role in motor control and execution, as highlighted by both microstimulation experiments and lesion studies (Alexander *et al.*, 1986). While the thalamus is also known to be an important centre for the transmission of sensory information, recent work has shown that the putamen also processes sensory information (Vicente *et al.*, 2012). Therefore, there may be two explanations for our results. First, it is possible that suppressed activity in these regions with baclofen was due to their high expression of $GABA_B$ receptors (Bowery *et al.*, 1987); however, this decrease in activity did not affect motor behaviour on a simple reaction time task, as a result of potential compensatory cortical or spinal mechanisms. The baclofen-related decreased activity in the primary motor cortex and premotor cortex after applying the pre-threshold masks suggests that the compensatory mechanisms have a spinal origin. Second, it is possible that the decrease in activity in these regions did not reflect only motor, but also sensory regions. Since baclofen is a muscle relaxer, less proprioceptive information could be arriving at the subcortical structures, leading to reduced activity compared to placebo. Indeed, striatal and thalamic GABA have been linked to proprioceptive mechanisms, with striatal GABA contributing to response speed, and thalamic GABA to regulating proprioceptive-induced response conflict (Dharmadhikari *et al.*, 2015).

4.4.2.4 Baclofen changed learning-related (sequence > random) patterns of brain activity

I investigated how learning-related brain activity (sequence > random) changed between baclofen and placebo sessions. The whole-brain analysis revealed higher learning-related activity in the following regions in the left hemisphere: primary motor cortex, posterior-middle temporal gyrus, angular gyrus, frontal orbital cortex and frontal pole during the baclofen session compared to placebo.

While these brain regions are not part of a clear network involved in motor learning, they are each known to contribute to related processes. Firstly, the primary motor cortex is responsible for encoding movement parameters, like force, direction and recruited muscles, as described in more detail in Chapter 1. M1 is also important for motor sequence learning, as both local changes in GABAergic inhibition and practice-related changes in M1 preparatory activity have been associated with motor sequence learning (Kolasinski *et al.*, 2019*a*; Hamano *et al.*, 2020). Secondly, the angular gyrus and the posterior middle temporal gyrus (pMTG), each part of the temporoparietal cortex, have been implicated together in semantic retrieval (Binder *et al.*, 2009). The angular gyrus is also a part of the default mode network, with roles in memory retrieval and attention; disrupting its activity using brain stimulation has been shown to affect automatic retrieval of semantic concepts (Seghier, 2013; Davey *et al.*, 2015). The pMTG shows activation during action observation (especially meaningful gestures), action feedback and creating novel associations; perhaps in our study, participants learnt to associate the abstract visual stimulus to the corresponding finger movement (van Kemenade *et al.*, 2019; Papeo *et al.*, 2019; Zhang *et al.*, 2020). Thirdly, the frontal pole and the frontal orbital cortex are

involved in numerous cognitive processes, including working memory, learning and response inhibition (Jonides *et al.*, 1998; Tsujimoto *et al.*, 2011; Baltz *et al.*, 2018). The role of the frontal pole and why it might be of particular importance in motor sequence learning has been discussed in more detail above. The frontal orbital cortex has rich connections with the sensory cortex, other areas of the prefrontal cortex and the thalamus, making it an important integration station that plays a role in predicting the outcomes of prospective decisions and using pre-learnt behaviour (Schreiner & Gremel, 2018; Rudebeck & Rich, 2018). Generally, the role of these areas is determined through neuroimaging studies reporting their activation during specific tasks, implying that higher activation serves the process in question. It is therefore difficult to explain why I found a higher learning-related activity in all these regions with baclofen concomitant with impairments on the motor learning task. This result might be a compensatory mechanism to the pharmacological increase in GABAergic inhibition. Alternatively, there might be more complex changes in the activity of these areas during motor learning; if the activity of these areas decreases once the motor sequence has been learnt, a higher level of activity would be seen with baclofen-related impairments.

After applying the mean SRTT activation mask as a pre-threshold mask, I only found a significant voxel cluster in the supplementary motor area (SMA) with higher learning-related activity with baclofen compared to placebo. The supplementary motor area is part of the motor network, having strong connections with the primary motor cortex and basal ganglia (Johansen-Berg *et al.*, 2004; Lehéricy *et al.*, 2004; Kim *et al.*, 2010). Therefore, the SMA is involved in motor control and motor learning (Nachev *et al.*, 2008; Brendel *et al.*, 2010): it has a somatotopic configuration highlighted by its spinal projections (Dum &

Strick, 1991; He *et al.*, 1995), its stimulation can evoke movement (Fried *et al.*, 1991) and invasive studies in primates show increased neuronal activity in a sub-region of the SMA during learning of an eye movement task (Chen & Wise, 1995). Increased activity in SMA and its rostral adjacent region, pre-SMA, has also been reported during motor learning in both primates and humans, likely reflecting the association of visual stimuli with motor responses (Hikosaka *et al.*, 1996; Nakamura *et al.*, 1998; Sakai *et al.*, 1999). Functional connectivity between SMA and subcortical areas like SMA-striatal connections is also behaviourally relevant: strong functional connectivity is associated with better performance on simple motor and cognitive tasks (Marchand *et al.*, 2012).

Stimulating the SMA using anodal tDCS leads to improvements in movement planning and motor learning (Vollmann *et al.*, 2013; Hupfeld *et al.*, 2017; Kim & Wright, 2020), although the low spatial specificity of tDCS means that other adjacent areas could have contributed to this effect. On the other hand, decreasing SMA and pre-SMA excitability with injected GABA agonists leads to impairments in motor sequence learning (Shima & Tanji, 1998; Nakamura *et al.*, 1999). Thus, modulating the activity of the SMA and the pre-SMA can change motor behaviour.

Here, I found impaired performance on a motor sequence learning task after administration of GABA agonist baclofen, but this behavioural change was associated with increased learning-related activity in the SMA compared to placebo. Considering the role of the SMA, I expected that any behavioural impairments would be associated with decreased activity in the SMA. The increased learning-activity in the SMA could be a compensatory mechanism for the increased GABAergic inhibition or it could reflect

increased learning-related activity in areas connected to the SMA which are not part of the SRTT network, like the prefrontal cortex (Wang *et al.*, 2005; Kim *et al.*, 2010).

4.4.3 Working memory was not significantly affected by baclofen

Learning new motor skills also requires working memory processes (Seidler *et al.*, 2012) and, as GABAergic inhibition plays an important role in working memory (Yoon *et al.*, 2016), I wanted to control for any baclofen-related effects on working memory that might affect motor sequence learning. Considering our healthy volunteers received a relatively low dose of baclofen, I hypothesised that there would be no effect of baclofen on simple CANTAB working memory tests. Indeed, I found no effect of baclofen on working memory capacity, spatial working memory, pattern recognition memory and attention, consistent with previous work in our lab (Johnstone *et al.*, 2021*b*). While animal literature reports baclofen-related impairments of working memory, these effects are dose-dependent and more pronounced at higher doses (Stackman & Walsh, 1994; Zarrindast *et al.*, 2002; HOLAJOVA & FRANEK, 2018). There are also reports of baclofen-induced improvements in working memory in animal models of brain injury and stress (Luo *et al.*, 2016; Sahraei *et al.*, 2019), where baclofen probably compensates for pathologically altered GABAergic signalling.

4.4.4 Baclofen did not significantly affect alertness or calmness, but it decreased participants' contentedness

Lastly, I studied how baclofen affected the participants' mood by comparing their alertness, calmness and contentedness scores from BLVAS between treatment conditions. Considering the relatively low dose of baclofen, I did not expect to see any significant effects of treatment on either of the mood components. Indeed, I saw no significant effect of treatment on alertness and calmness, but I found a significant effect of time on alertness; this was likely due to participants getting more tired throughout the 4-hour testing session and after the MRI scan. Somnolence can be a side-effect of baclofen, but it usually occurs at doses higher than the one administered here (Rigal *et al.*, 2015; Ertzgaard *et al.*, 2017). I also found a significant effect of treatment on contentedness: participants were less content during the baclofen session compared to placebo. Previous literature investigating the effect of baclofen shows the opposite: an anxiolytic role for baclofen has been reported when administered to patients with addictions (Krupitsky *et al.*, 1993; Addolorato *et al.*, 2002; Garbutt *et al.*, 2010; Morley *et al.*, 2014), but null findings have also been reported (Farokhnia *et al.*, 2017). However, I found no previous studies reporting decreases in contentedness with baclofen. It is possible that this decrease in contentedness is a result of participants' experiencing undisclosed side-effects or being aware of their impaired performance on the motor sequence tasks.

4.5 Conclusion

In this chapter, I investigated the effect of increasing GABAergic signalling *via* baclofen on brain activity during performance of motor tasks. I found significant differences in the patterns of brain activity during the SRTT task between treatment conditions, notably higher activity in the frontal pole during sequence learning and lower activity in the thalamus and basal ganglia with baclofen. The areas with increased activity during motor learning in the baclofen session are likely recruited as a compensatory mechanism and could potentially be used as targets for brain stimulation, to aid motor performance in neurorehabilitation trials.

CHAPTER 5 - THE EFFECT OF BACLOFEN ON RESTING-STATE FUNCTIONAL CONNECTIVITY

In Chapter 3, I explored the effects of modulating GABAergic inhibition on motor learning and brain chemistry and reported that pharmacologically increasing GABAergic inhibition using baclofen led to impairments in performance of a motor sequence learning task. Motor behaviour relies on the correct activation of the sensorimotor network. Motor learning is associated with behaviourally relevant functional connectivity changes in the motor network at rest (Sampaio-Baptista et al., 2015). Functional connectivity is linked to GABAergic inhibition in the motor cortex, with higher functional connectivity being associated with lower levels of M1 [GABA] (Stagg *et al.*, 2014). *GABAergic inhibition, connectivity in the motor network and motor learning are therefore closely related and network connectivity might be the link between the increased GABAergic inhibition and the behavioural impairments I reported in Chapter 3. I hypothesised that increasing GABAergic inhibition would be associated with decreased functional connectivity in resting-state networks, which would be correlated with [GABA] in motor areas.*

5.1 INTRODUCTION

5.1.1 Resting-state networks

Even at rest, the brain is still active and physiological fluctuations in the activity of different brain areas can be measured using resting-state fMRI. Analysis of resting-state fMRI reveals resting-state networks (RSN): functionally connected brain areas with similar patterns of activity across time (Biswal *et al.*, 1995; Damoiseaux *et al.*, 2006). These RSNs are similar to task-based networks and have been linked to behavioural domains, e.g. the sensorimotor network to motor execution and somaesthetic perception, the auditory

network to auditory perception and action execution of speech, and the executive control network to action inhibition and cognition (Smith *et al.*, 2009*a*; Laird *et al.*, 2011). This similarity supports the hypothesis that both task-based and resting-state networks rely on intrinsic anatomical connectivity (Cole *et al.*, 2014; Gratton *et al.*, 2018); indeed, connectivity between regions of interest (ROIs) in resting-state networks is significantly correlated with their connectivity in task-based networks (Di *et al.*, 2013) and resting-state data can predict individual variability in task-based networks (Tavor *et al.*, 2016).

Moreover, functional connectivity strength derived from resting-state networks has been shown to correlate with performance on several tasks, like visuomotor associative learning task, n-back task and spatial attention task (Sala-Llonch *et al.*, 2012; Kim & Kang, 2018; Machner *et al.*, 2021; Li *et al.*, 2022), stronger functional connectivity being generally associated with better task performance. Of particular relevance here, metrics of resting-state functional connectivity pre-learning are correlated with motor learning metrics in both the fMRI-derived RSNs (Bonzano *et al.*, 2015) and the MEG/EEG-derived RSNs (Wu *et al.*, 2014; Mary *et al.*, 2017; Sugata *et al.*, 2020). Functional connectivity is also known to change with motor learning and these changes correlate with task performance metrics (Sampaio-Baptista *et al.*, 2015).

5.1.2 The role of GABA in functional connectivity

GABA-mediated inhibition contributes to network homeostasis, as GABAergic neurons play a critical role in generating and propagating network oscillations; at a cellular level, interneurons control spike timing in excitatory neurons and interact with other intra-network interneurons (Mann & Paulsen, 2007; Roth & Draguhn, 2012), while, at a network level, M1 [GABA] has been significantly correlated with the frequency and power

of event-related synchronisation in the gamma band, as measured with MEG (Gaetz *et al.*, 2011). Further evidence supporting the link between GABA and network connectivity is provided by resting-state fMRI studies in humans (Kapogiannis *et al.*, 2013; Stagg *et al.*, 2014; Bachtiar *et al.*, 2015; Levar *et al.*, 2019). Specifically, functional connectivity in the motor network is negatively correlated with M1 [GABA] (Stagg *et al.*, 2014), as is connectivity in the default mode network and GABA levels in the posteromedial cortex (Kapogiannis *et al.*, 2013). Moreover, when learning a new motor task, the learning-related changes in functional connectivity in the motor network are correlated with both baseline M1 [GABA] (King *et al.*, 2020) and the learning-related changes in M1 [GABA] (Sampaio-Baptista *et al.*, 2015).

5.1.3 Aim

I conducted a within-subject, double-blind, placebo-controlled study to investigate the effect of a single dose of baclofen (20mg) on functional connectivity. **I hypothesised that baclofen would decrease the functional connectivity of resting-state networks and aimed to replicate previous findings showing that functional connectivity in the motor network was negatively correlated with pre-task M1 [GABA]. I hypothesised that functional connectivity in the motor network would also be correlated with pre-task [GABA] across the motor network.**

5.2 METHODS

5.2.1 Experimental design

As previously described in Chapter 3, I recruited 18 young, healthy volunteers to take part in this study. All volunteers participated in two sessions at least one week apart; the sessions were identical except on one they received a dose of 20mg of baclofen and, on the other, they received a placebo pill (sucrose/lactose). During each session, participants had an MRI scan, which included a resting-state fMRI scan. The resting-state scan took place before the MRSI scans and the task fMRI scans. Three participants could not complete the MRI scans due to claustrophobia, so all further analyses only included data from 15 participants.

5.2.2 MRI Data Acquisition

All MRI data were acquired in a 3T Siemens Magneton Scanner using a 32-channel head coil (Nova Medical Inc, Wilmington, USA), as described in Chapter 3. The T1-weighted structural scan was followed by a resting-state fMRI scan, matched to the resting-state UK Biobank parameters (Alfaro-Almagro *et al.*, 2018): T2*-weighted echo-planar imaging (EPI) sequence, TR = 0.735s, TE = 39ms, slice thickness = 2.40mm, TA = 6.16min. During the resting-state scan, participants were asked to stay awake, keep their eyes open and look at a fixation cross on the screen. Then, the pre-task MRSI scan was acquired (semi-LASER DW-CRT sequence with metabolite cycling (Steel *et al.*, 2018), TR = 1.4s, TE = 32ms, TA = 2 x 4.5min, voxel size = 5 x 5 x 15 mm^3). Two task fMRI scans were then acquired using a T2*-weighted EPI sequence, as described in Chapter 4. Lastly, a post-task MRSI scan was acquired. MRSI data acquisition, processing and analysis were described in more detail in Chapter 3.

After the acquisition, all resting-state data were organised following the Brain Imaging Data Structure (Gorgolewski *et al.*, 2016).

5.2.3 Resting-state fMRI analysis

5.2.3.1 Lower-level analysis

fMRI data were processed using the Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC), which is included in the FMRIB Software Library (FSL) (Jenkinson *et al.*, 2012*b*). Pre-processing for the single-session analysis included motion correction using MCFLIRT (Jenkinson *et al.*, 2002*b*), brain extraction and bias-field correction using fsl_anat, which included the FSL Brain Extraction Tool and FMRIB's Automated Segmentation Tool (Zhang *et al.*, 2001; Smith, 2002), fieldmap-based EPI unwarping, and high-pass temporal filtering equivalent to 100.0s. The single-session MELODIC output was cleaned using FMRIB's ICA-based Xnoiseifier (FIX) and UK Biobank training data (Salimi-Khorshidi et al., 2014; Griffanti et al., 2014). Spatial smoothing with a Gaussian kernel of FWHM 5.0 mm was then used on the clean data. The smoothed and clean functional data were then registered to standard space using the linear and non-linear registration parameters from the MELODIC output and these registered data were used in further analysis.

5.2.3.2 Higher-level analysis

Single-session registered functional data were temporally concatenated across subjects to create a single 4D dataset, which was analysed using a dual regression technique (Nickerson et al., 2017). First, 18 components were identified using ICA on the concatenated dataset and resting-state networks (RSNs) of interest were distinguished by

spatial cross-correlation against previously described networks (Smith *et al.*, 2009*b*). Dual regression was then used to determine the subject-specific RSN maps. These resulting maps were masked by the group mean RSN map and the mean value within this region was extracted for each subject, to be used as a measure of functional connectivity strength within the network.

Three RSNs were further investigated: sensorimotor network, visual network and default mode network. I selected these networks, as their activity relates to the performance of the tasks employed. Specifically, the sensorimotor network includes areas responsible for motor control and motor learning; the visual network includes areas implicated in detecting and processing visual stimuli; the default mode network is deactivated during task performance, likely due to the reallocation of attention and cognitive processes toward the task (Fox *et al.*, 2005; Buckner *et al.*, 2008; Singh & Fawcett, 2008; Smith *et al.*, 2009*a*).

To compare the functional connectivity of these networks between baclofen and placebo conditions, I performed a voxel-wise analysis using a paired test design in FSL's tool randomise with Threshold-Free Cluster Enhancement and cluster-based threshold outputs for Z values over 3.1 and p values < 0.05 FEW-corrected (Winkler et al., 2014). Within-network functional connectivity was assessed using the group RSN map as a pre-threshold mask, while whole-brain functional connectivity of the network was assessed using the same analysis without a pre-threshold mask.

To probe the relationship between functional connectivity and behaviour, I performed correlation analyses between the strength of the sensorimotor network and behavioural

metrics: measurements of response times during the motor tasks and the SRTT learning metrics as described in Chapter 3.

5.2.4 Statistical analysis

Both the analysis of mean connectivity strength and correlations with behavioural and [GABA] metrics were performed using Prism (v.9.3.0, GraphPad Prism Software). Analysis was performed using repeated-measures ANOVA or paired t-tests with significance levels of α=0.05 to investigate the within-subject effects of baclofen compared to placebo. Relationships between functional connectivity and either behavioural metrics or [GABA] measurements were investigated by calculating Pearson's correlation coefficients. Graphs show individual data points unless otherwise indicated.

5.3 Results

5.3.1 Baclofen significantly affected the strength of resting-state networks

In order to evaluate the effect of baclofen on functional connectivity, I first identified group-level resting-state networks (RSNs) and focused on three networks of interest: sensorimotor, visual and default mode network (Fig. 5.1).

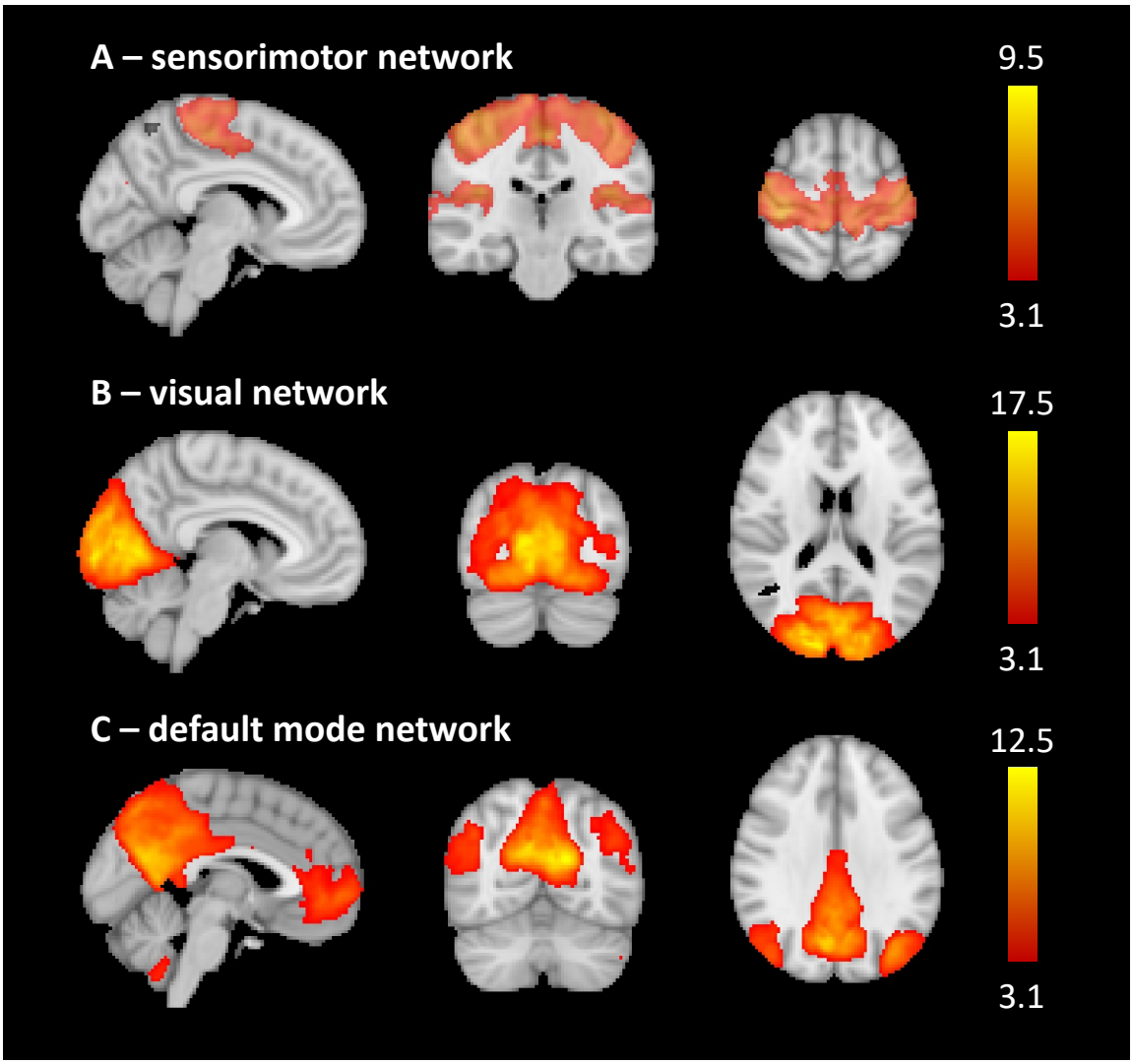


Fig. 5.1. **Resting-state networks of interest: sensorimotor (A), visual (B) and default mode network (C).** These masks were then used as pre-threshold masks in resting-state group-level analyses

I then calculated the mean network strength for each network and studied how this value changed between treatment conditions. I used a repeated-measures two-way ANOVA with within-subject factors of treatment (baclofen, placebo) and network (motor, visual, DMN) and found a significant effect of network ($F(1.613,22.58) = 5.757$, $p = 0.0135$), treatment ($F(1, 14) = 5.608$, $p = 0.0328$) and a significant network x treatment interaction ($F(1.368, 19.15) = 4.107$, $p = 0.046$; Fig. 5.2). A post-hoc Bonferroni's multiple comparisons test showed significantly decreased network strength in the visual network with baclofen ($t(14) = 2.73$, *adjusted* $p = 0.049$), but not in the sensorimotor network ($t(14) = 1.78$, *adjusted* $p = 0.29$) or the DMN ($t(14) = 0.281$, *adjusted* $p > 0.99$).

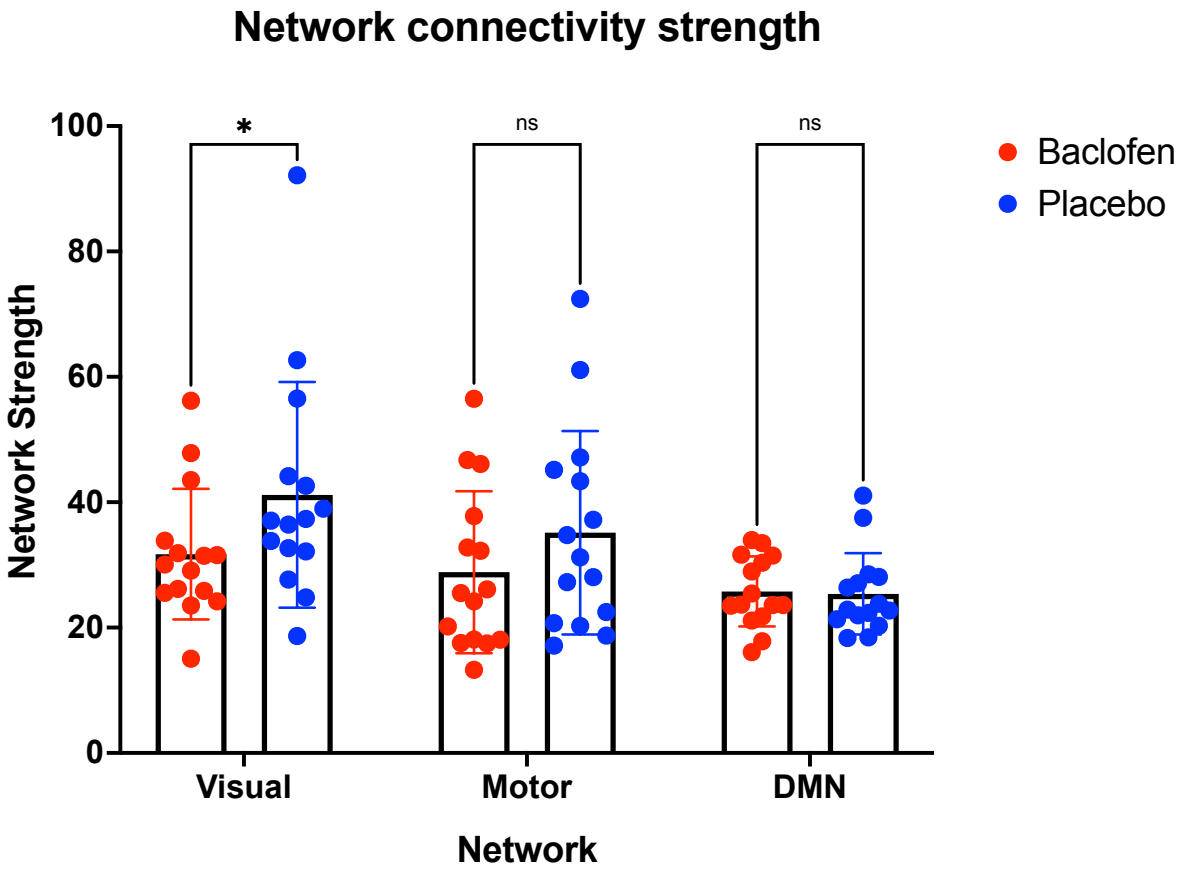


Fig. 5.2. **Baclofen significantly affected resting-state network connectivity strength**. Individual data points represent each participant's mean network connectivity strength. Columns and bars represent mean +/-SD. * $p < 0.05$ corrected for multiple comparisons

5.3.2 Baclofen decreased within-network connectivity in the sensorimotor and the visual network

To further examine the effect of baclofen on functional connectivity, I conducted a voxel-wise analysis to detect which brain areas had significantly decreased within-network functional connectivity after baclofen administration compared to placebo. I found decreased functional connectivity in the right primary somatosensory cortex in the sensorimotor network, specifically in Brodmann areas (BA) 1 and 3b (Fig. 5.3A). In the visual network, I found decreased connectivity in the V3V region, lingual gyrus, occipital fusiform gyrus and the lateral occipital cortex (Fig. 5.3B). No significant results were found in the DMN.

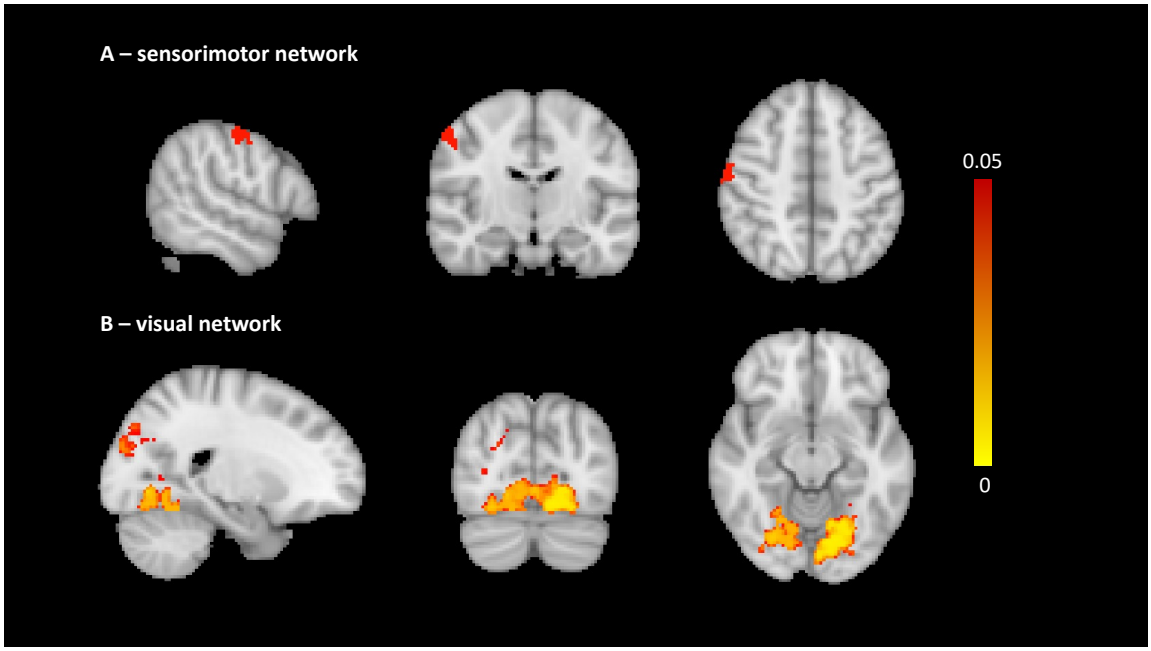


Fig. 5.3. **Areas with decreased connectivity in the sensorimotor network (A) and visual network (B)**. Colour scale represents significant p values from 0 to 0.05

5.3.3 Higher motor network strength was associated with higher response times with baclofen

I then investigated how functional connectivity in the sensorimotor network related to behavioural metrics. I found a significant positive correlation between motor network strength and response times during execute blocks of the action-selection task during the baclofen session ($r(12) = 0.565$, $p = 0.035$), but not placebo ($r(12) = 0.246$, $p = 0.397$; Fig. 5.4A). I found no significant correlations between motor network strength and response times during random blocks of the SRTT (baclofen: $r(12) = 0.061$, $p = 0.835$; placebo: $r(12) = 0.161$, $p = 0.583$; Fig. 5.4B) or between motor network strength and the SRTT learning metric (baclofen: $r(12) = -0.097$, $p = 0.741$; placebo: $r(12) = -0.197$, $p = 0.499$; Fig. 5.4C).

Lastly, I found no significant correlation between the skill acquired during motor learning (R2 – S11) and motor network strength (baclofen: $r(12) = -0.067$, $p = 0.819$; placebo: $r(12) = -0.254$, $p = 0.381$).

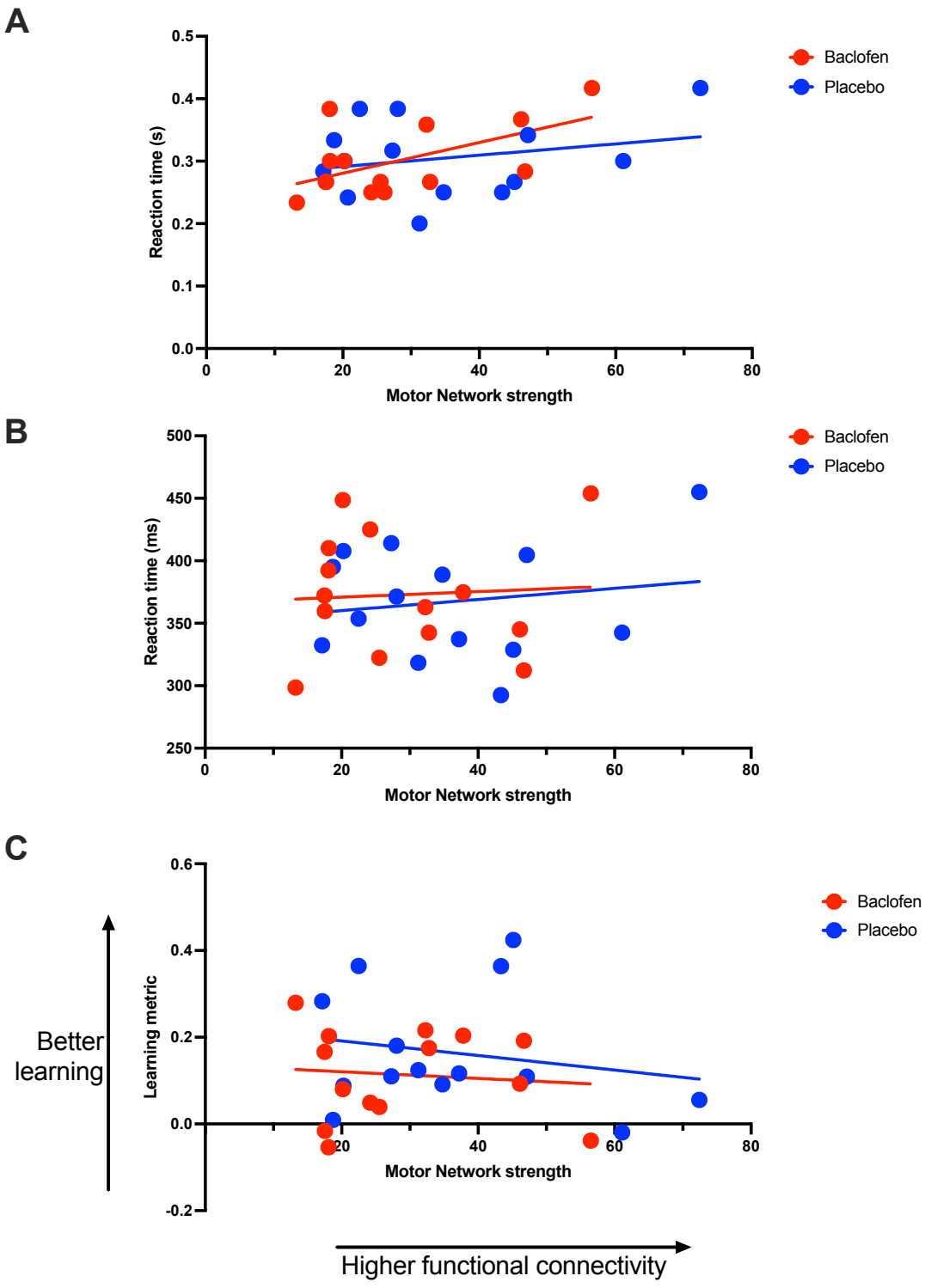


Fig. 5.4. **Relationship between motor network strength and behavioural metrics**. (A) Motor network strength and response time during execute blocks in action-selection task; (B) Motor network strength and response time during random blocks in SRTT; (C) Motor network strength and SRTT learning metric

5.3.4 Baclofen did not significantly affect pre-task [GABA] in the sensorimotor network

I then investigated how baclofen changed [GABA] in the sensorimotor network. For each participant, I identified the voxels from the GABA:Cre maps (as described in Chapter 3) that overlapped with the participant's sensorimotor network (Fig. 5.5) and calculated the mean [GABA] value in these voxels.

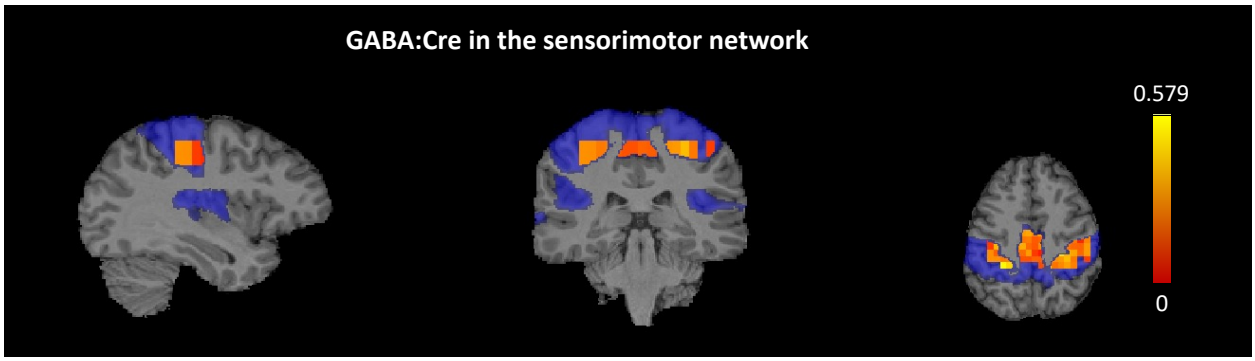


Fig. 5.5. **Example of GABA: Cre map in the sensorimotor network in one participant**

I compared sensorimotor [GABA] between treatment conditions and found that baclofen did not significantly change sensorimotor [GABA] (paired t-test $t(14) = 0.348$, $p = 0.733$; Fig. 5.6A).

5.3.5 No significant correlations between motor network strength and pre-task [GABA] in motor regions

I then investigated whether MRSI-derived measurements of GABA in the sensorimotor network or motor regions were correlated with motor network strength. I found no significant correlation between sensorimotor [GABA] and motor network strength (baclofen: $r(13) = -0.095$, $p = 0.735$; placebo $r(13) = 0.080$, $p = 0.776$; Fig. 5.6B), left M1 [GABA] and motor network strength (baclofen: $r(13) = 0.002$, $p = 0.993$; placebo $r(13) =$

0.094, p = 0.738; Fig. 5.6C) or left premotor cortex [GABA] and motor network strength (baclofen: $r(13)$ = -0.205, p = 0.463; placebo $r(13)$ = 0.342, p = 0.212; Fig. 5.6D).

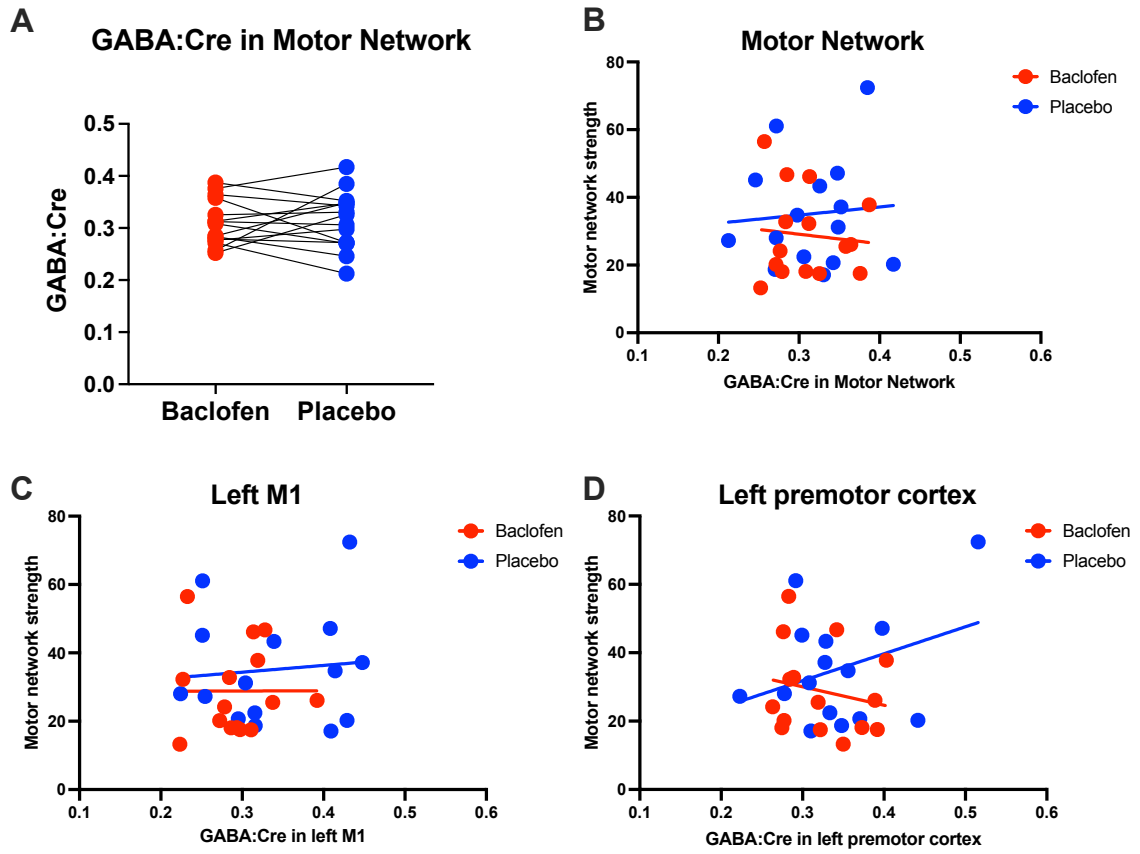


Fig. 5.6. **Pre-task [GABA] in the sensorimotor network**. (A) Sensorimotor pre-task [GABA] is not significantly different between treatment conditions; (B) No significant correlation between sensorimotor pre-task [GABA] and motor network strength; (C) No significant correlation between left M1 pre-task [GABA] and motor network strength; (D) No significant correlation between left premotor cortex pre-task [GABA] and motor network strength

5.4 Discussion

To investigate the role of GABAergic inhibition in functional connectivity, I studied the effect of baclofen on resting-state networks. I found that baclofen significantly decreased functional connectivity in resting-state networks and voxel-wise analysis showed the clusters with significantly lower within-network connectivity in the sensorimotor and visual networks. I found no significant correlation between sensorimotor network strength and either behaviour or sensorimotor [GABA].

5.4.1 The effect of baclofen on resting-state functional connectivity

5.4.1.1 Baclofen was associated with decreased functional connectivity in RSNs

I then investigated the effect of baclofen on resting-state functional connectivity by focusing on three resting-state networks (RSNs) relevant to task performance; the sensorimotor network includes areas responsible for motor control and motor learning, the visual network includes areas implicated in detecting and processing visual stimuli and the default mode network is deactivated during task performance, likely reflecting the reallocation of attention and cognitive processes towards the task (Fox *et al.*, 2005; Buckner *et al.*, 2008; Singh & Fawcett, 2008; Smith *et al.*, 2009*a*).

GABAergic inhibition is known to be related to resting-state functional connectivity, with stronger functional connectivity being associated with lower [GABA] (Kapogiannis *et al.*, 2013; Stagg *et al.*, 2014; Levar *et al.*, 2019; Chen *et al.*, 2019), but null results have also been reported (Gonen *et al.*, 2020). Moreover, decreasing GABAergic inhibition using $GABA_A$-receptor antagonist bicuculine led to increased functional connectivity in

anaesthetised rats and increased EEG coherence in the alpha and beta bands (Nasrallah *et al.*, 2017). Therefore, I hypothesised that increasing GABAergic inhibition via baclofen would lead to decreased functional connectivity in RSNs. Indeed, I found an overall effect of treatment on the three RSNs, with baclofen decreasing functional connectivity compared to placebo. Post-hoc analysis showed a significant decrease in functional connectivity in the visual network after correcting for multiple comparisons, but no significant effect in the other two networks. This effect of baclofen on functional connectivity may be mediated by changes in oscillatory activity, as GABAergic inhibition is of particular importance in generating brain oscillations (see Chapter 1) and pharmacologically disrupting GABAergic signalling may lead to desynchronisation and subsequent decrease in functional connectivity. While baclofen has been shown to change TMS-induced oscillatory responses in the alpha and beta bands, it is still unclear how it affects TMS-induced brain oscillations in other frequency bands or at rest (Premoli *et al.*, 2017).

These results are in contrast with the effect of $GABA_A$-receptor agonists on functional connectivity; there is strong evidence that $GABA_A$-receptor agonists (alcohol, zolpidem, diazepam, alprazolam) increase resting-state functional connectivity, especially in visual and sensorimotor cortices (Esposito *et al.*, 2010; Licata *et al.*, 2013; Pflanz *et al.*, 2015; Blanco-Hinojo *et al.*, 2021). This effect is hypothesised to occur due to pharmacologically-induced increases in power in the beta and gamma bands after administration of $GABA_A$ agonists (van Lier *et al.*, 2004; Jensen *et al.*, 2005*b*; Hall *et al.*, 2010; de Haas *et al.*, 2010), which synchronises the activity of neuronal populations and increases functional connectivity (Fingelkurts *et al.*, 2004). These changes are mapped to the spatial

distribution of $GABA_A$ receptors (Hall *et al.*, 2010; Shaw *et al.*, 2021), which potentially explains why I did not find a similar effect after the administration of a $GABA_B$ receptor agonist. Other possible explanations for these conflicting results include differences in data acquisition (eyes-closed versus eyes-open during resting-state acquisition (Agcaoglu *et al.*, 2019)), longer-term administration of the drug (7-day diazepam) (Pflanz *et al.*, 2015) or recruiting participants with underlying conditions (social anxiety) (Blanco-Hinojo *et al.*, 2021).

5.4.1.2 Voxel-wise analysis revealed areas of decreased within-network connectivity with baclofen

To investigate whether specific brain areas showed decreased within-network functional connectivity after baclofen administration, I conducted a voxel-wise analysis to compare the activity of the 3 RSNs of interest between treatment conditions. With baclofen, I found areas of decreased functional connectivity in both the visual network (ventral region of the V3 area, V2, V4 and lateral occipital cortex) and the sensorimotor network (right BA1 and BA4a regions).

$GABA_B$ receptors are widely expressed throughout the brain and in most neuronal cell types (Bischoff *et al.*, 1999; Billinton *et al.*, 2000); in visual areas, $GABA_B$ receptors have a high expression in the supragranular layer (Eickhoff *et al.*, 2007). Extrastriate visual areas (V2-V4) are associative visual areas, implicated in gradually more complex, integrative processes; for example, V2 has been reported to encode combinations of orientations, while V3 and V4 are more heterogeneous and have been implicated in encoding colour, motion processing, spatial attention before sending this information to higher-order areas (Anzai *et al.*, 2007; ARCARO & KASTNER, 2015). GABAergic inhibition plays an

important role in visual processes: interneurons can be tuned to the orientation of visual stimuli (Runyan & Sur, 2013), visual [GABA] correlates with performance on a visual orientation discrimination task (Edden *et al.*, 2009) and learning-related changes in visual [GABA] correlate with behavioural improvements on visual tasks (Frangou *et al.*, 2019). Considering the presence of $GABA_B$ receptors in the visual network and the important role of GABAergic inhibition for vision, it is not surprising that pharmacologically modulating GABAergic inhibition affected the visual network widely. Additionally, I asked participants to keep their eyes open and look at a fixation cross during resting-state data acquisition; therefore, the underlying visual activity may have highlighted the baclofen-related decreases in functional connectivity in this network more than in other RSNs.

Regions in the right primary somatosensory and motor cortices also showed decreased functional connectivity with baclofen. $GABA_B$ receptors are expressed in the sensorimotor network (Bowery *et al.*, 1987; Billinton *et al.*, 2000) and I have discussed in more detail the role of GABAergic inhibition in motor control and motor learning above. The primary somatosensory and motor cortex are also active during motor imagery (Porro *et al.*, 1996; Jafari *et al.*, 2020); despite our instructions, the participants could have imagined motor behaviour, so I cannot exclude that the sensorimotor network was activated by imagined movements. However, the areas of decreased functional connectivity are quite small and peripheral, which means they could potentially be a false-positive result.

5.4.1.3 The relationship between functional connectivity and behaviour

I then investigated the relationship between functional connectivity in the sensorimotor network and motor behaviour. I found no significant correlation between network strength and response times (either execute response times from the AS task or random

response times from the SRTT) during the placebo session, but I found a significant positive correlation between motor network strength and execute response time during the baclofen session.

The strength of functional connectivity in the corresponding network is generally positively correlated with performance on visuomotor and cognitive tasks (Sala-Llonch *et al.*, 2012; Kim & Kang, 2018; Machner *et al.*, 2021; Li *et al.*, 2022). The relationship between functional connectivity and simple response times is still incompletely understood (Siettos & Smyrnis, 2017). It was previously thought that the response times were the sum of the duration required for electric conduction through the corticospinal tract and then spinal projections, but response times show within-subject variability, which indicates that other mechanisms like oscillatory activity and network activity are likely involved (Hanes & Schall, 1996; Schall, 2001; Prado *et al.*, 2011; Zhou *et al.*, 2012; Paraskevopoulou *et al.*, 2021). However, to date, there is no evidence of significant correlations between fMRI-derived measures of sensorimotor connectivity and response times, potentially due to low temporal resolution for fMRI compared to EEG/MEG and the fact that the fMRI-derived sensorimotor network usually does not include subcortical motor regions, like the cerebellum and basal ganglia. It is difficult to explain why I only found a significant correlation between functional connectivity and response times during the baclofen session, especially when brain activity patterns during execute blocks were not significantly different between treatment conditions. It is possible this result arose from the effect of baclofen on spinal circuits not investigated here.

I also found no correlation between motor network strength and motor sequence learning. There are previous studies that show a relationship between functional

connectivity and motor learning, but both fMRI- and MEG/EEG connectivity metrics in these studies are seed-based and therefore do not include the entire sensorimotor network, as our analysis did (Wu *et al.*, 2014; Bonzano *et al.*, 2015; Mary *et al.*, 2017; Sugata *et al.*, 2020; Baldassarre *et al.*, 2021). The participants' learning rate in a juggling task correlated with learning-related changes in sensorimotor functional connectivity, but not with the pre-learning functional connectivity (Sampaio-Baptista *et al.*, 2015). It is possible that if I acquired resting-state data after the motor sequence task, I would be able to show similar findings. It is also possible that, by reducing the dimensionality of the data from time series of individual voxels to the mean network strength, we lose spatial and temporal information about within-network functional connectivity, which may mediate motor behaviour and learning. Indeed, one study investigating the link between GABA, functional connectivity and sensorimotor performance showed that sensorimotor network segregation (increased within-network and decreased between-network connectivity) mediated the effect of sensorimotor [GABA] on behavioural metrics (Cassady *et al.*, 2019).

5.4.2 Sensorimotor [GABA] was not significantly affected by baclofen and did not correlate with sensorimotor network strength

Considering the reported negative correlation between M1 [GABA] and motor network strength (Stagg *et al.*, 2014; Bachtiar *et al.*, 2015), I set out to investigate the relationship between [GABA] in the sensorimotor network (sensorimotor [GABA]) and motor network strength. I hypothesised that I would replicate the previously reported finding and also find a negative correlation between sensorimotor [GABA] and motor network strength. Contrary to our hypothesis, I found no significant relationship between [GABA] in any of

the ROIs (motor network, M1, premotor cortex) and motor network strength in either the baclofen or the placebo sessions. This may be explained by differences in data acquisition parameters and spatial resolution (one MRS voxel of 8 cm^3 was used in previous studies versus 96 MRSI voxels of 375mm^3 used here). GABA concentrations are different between grey and white matter (Jensen *et al.*, 2005*a*), so the different sizes of the voxels, which inherently include both grey and white matter, might affect the level of the reported neurochemical. There are also differences in reporting neurochemical concentrations between studies (GABA/NAA in Stagg et al., 2014a versus GABA/tCr here). I decided to use total creatine as a reference because its concentration in the brain is relatively stable under physiological conditions (Rackayova *et al.*, 2017). While NAA is also a neurochemical that can be reliably detected using MRS, its numerous roles in neuronal activity (mitochondrial energy production, myelination, osmoregulation) make it a less reliable reference (Moffett *et al.*, 2007).

I also found no effect of baclofen on sensorimotor [GABA], which is consistent with the results I found in the M1 and premotor cortex. As discussed in more detail in Chapter 3, baclofen acts on both pre- and post-synaptic $GABA_B$ receptors (Benarroch, 2012), so its effect on total [GABA] depends on the proportion of pre-and post-synaptic receptors and is difficult to predict. Moreover, MRS is known to measure the total [GABA] in the voxel of interest on a timescale of several minutes, which does not reflect synaptic activity (Stagg *et al.*, 2011*b*; Tremblay *et al.*, 2013; Mooney *et al.*, 2017). Instead, MRS has been hypothesised to be a measurement of extrasynaptic, tonic inhibition (Stagg *et al.*, 2011*b*; Stagg, 2014), which baclofen might not affect, despite increasing GABAergic signalling via $GABA_B$ receptors.

5.5 Conclusion

In this chapter, I investigated the effect of increasing GABAergic signalling *via* baclofen on functional connectivity at rest. I found that baclofen significantly decreased functional connectivity in resting-state networks, with specific areas in both the visual and sensorimotor networks showing decreased within-network connectivity. Sensorimotor [GABA] did not significantly correlate with the motor network strength and was not changed by baclofen administration.

CHAPTER 6 - THE EFFECT OF ZOLPIDEM ON MOTOR LEARNING AND TMS METRICS

In the previous two chapters, I investigated the role of $GABA_B$ receptors in motor learning using baclofen, a specific $GABA_B$ receptor agonist, as a pharmacological intervention. However, the effects of GABAergic inhibition are mediated by both $GABA_A$ and $GABA_B$ receptors and the link between GABAergic inhibition and motor learning has not yet been shown to be receptor specific. Here, I probed the contribution of $GABA_A$ receptors to motor learning and touch perception using a pharmacological intervention, sensorimotor tasks and transcranial magnetic stimulation (TMS). I conducted a within-subject, double-blind, placebo-controlled study where I investigated the effect of zolpidem, a $GABA_A$ receptor positive allosteric modulator, on motor learning, touch perception and cortical excitability. I hypothesised that, by increasing $GABA_A$-mediated inhibition, zolpidem would impair motor learning, improve tactile discrimination and change TMS metrics of GABAergic inhibition in young, healthy volunteers. This question is clinically relevant: zolpidem is commonly prescribed as a sleeping pill to clinical populations including stroke survivors, potentially impairing their motor rehabilitation.

6.1 INTRODUCTION

6.1.1 The role of GABAergic inhibition in motor learning

As described in more detail in Chapter 1, GABAergic inhibition is known to play an important role in motor learning and plasticity in humans: M1 [GABA] decreases during motor sequence learning (Floyer-Lea *et al.*, 2006; Kolasinski *et al.*, 2019*b*) and NIBS-induced decreases in M1 [GABA] correlate with better motor learning (Stagg *et al.*,

2011*a*). However, these studies use MRS-derived metrics of [GABA] which quantify the total [GABA] in the volume of interest, with no information on receptor specificity or signalling pathways. Administration of both $GABA_A$ and $GABA_B$ receptor agonists has been shown to suppress LTP-like processes in M1 (McDonnell *et al.*, 2007; Heidegger *et al.*, 2010; Ziemann *et al.*, 2015), but the contribution of each receptor type to motor learning and behavioural outcomes is still unclear. Having studied the role of $GABA_B$ receptors in the previous chapters, I set out to investigate the role of $GABA_A$ receptors here, hypothesising that increasing $GABA_A$-mediated inhibition would impair motor learning.

6.1.2 The role of GABAergic inhibition in tactile discrimination

GABAergic inhibition is also implicated in somatosensory processing: higher sensorimotor [GABA] is associated with better performance in tactile discrimination tasks in both healthy people and clinical populations (Puts *et al.*, 2011; Kolasinski *et al.*, 2017; Sapey-Triomphe *et al.*, 2019). Moreover, higher baseline sensorimotor [GABA] is associated with better learning in a tactile perception task (Heba *et al.*, 2016). The underlying mechanism is hypothesised to be related to the excitatory signal becoming more spatially focused by the surrounding inhibitory processes, leading to better sensory discrimination. Whether modulating GABAergic inhibition leads to changes in tactile discrimination is still unclear. One study showed impaired performance in a tactile perception task after administration of $GABA_A$ receptor positive allosteric modulator alprazolam (Fioravanti *et al.*, 2019); increased inhibition was associated with longer response times and higher variability in response times in the tactile perception task, but changes in tactile discrimination were not measured. Here, I hypothesised that increasing GABAergic inhibition would lead to improvements in tactile discrimination.

6.1.3 Zolpidem – mechanism of action and clinical use

Zolpidem is a selective non-benzodiazepine positive allosteric modulator for the α1 subunit of the $GABA_A$ receptor (Crestani et al., 2000; Möhler, 2010). $GABA_A$ receptors are pentameric ligand-gated channels, which combine 5 subunits around a central pore permeable for chloride ions; due to the low intracellular chloride concentration in adult neurons, the opening of the $GABA_A$ receptors leads to chloride influx and subsequent cellular hyperpolarisation (Farrant & Nusser, 2005).

There are 8 main categories of $GABA_A$ subunits, with different subunit compositions: α(1-6), β(1-3), γ(1-3), $\delta, \varepsilon, \theta, \pi, \rho$(1-3) (Sigel & Steinmann, 2012), which are associated with distinct gating and pharmacokinetic properties (Engin *et al.*, 2018; Castellano *et al.*, 2021). The pentamer containing $\alpha 1 \beta 2 \gamma 2$ is the most commonly expressed $GABA_A$ receptor, known to mediate sedative and anticonvulsant clinical effects (Möhler *et al.*, 2004). $GABA_A$ α1 subunits have a high expression across all brain structures, especially in the cerebral and cerebellar cortex (Sequeira *et al.*, 2019). Zolpidem potentiates GABAergic inhibition by increasing the duration of the α1-containing $GABA_A$ receptor activation and enhancing GABA currents (Perrais & Ropert, 1999; Chen *et al.*, 2007; Dixon *et al.*, 2015).

Zolpidem is safe to administer to humans and it is commonly used as a hypnotic in the treatment of insomnia in both healthy people and clinical populations (Dang *et al.*, 2010; Castro *et al.*, 2020); zolpidem has also been trialled for disorders of consciousness and movement disorders (Bomalaski *et al.*, 2017). When administered orally, it crosses the blood-brain barrier and it reaches peak plasma concentration in approximately 1.5 hours; it is then hepatically metabolised, with a half-life of approximately 2.2-2.5 hours (Dang *et*

al., 2010; de Haas *et al.*, 2010). According to the British National Formulary, the recommended oral dose is 10mg at bedtime, although elderly patients may only receive 5mg; common side effects include dizziness, fatigue, nausea and headaches (BNF, n.d.).

6.1.4 The effect of Zolpidem on TMS metrics

Zolpidem has been safely used in previous brain stimulation studies investigating inhibitory circuits in motor brain areas (Mohammadi *et al.*, 2006; Di Lazzaro *et al.*, 2006, 2007; Teo *et al.*, 2009). One study showed an increase in the TMS metric of $GABA_A$-mediated inhibition (short-interval intracortical inhibition, SICI) with zolpidem at 1h after administration compared to baseline, although the intensity of the test pulse was not adjusted to match the amplitude of the test MEP between measurements (Mohammadi *et al.*, 2006). However, numerous studies provide evidence that one single dose of 10 mg of zolpidem does not affect SICI at 2h after treatment administration, unlike non-subunit specific benzodiazepines (Di Lazzaro *et al.*, 2006, 2007; Teo *et al.*, 2009). This indicates that SICI is likely mediated by $GABA_A$ receptors containing subunits α2 or α3, but not α1. Measurements of cortical excitability like resting motor threshold (rMT) and active motor threshold (aMT) also do not change with zolpidem (Di Lazzaro *et al.*, 2006; Teo *et al.*, 2009). However, these studies did not have a placebo arm, and the analysis was performed by comparing post-treatment time points to the baseline time point. Here, I investigated the effect of a smaller dose of zolpidem (5mg) on TMS metrics of cortical excitability and $GABA_A$-mediated inhibition compared to placebo.

6.1.5 The effect of Zolpidem on motor behaviour

Zolpidem is known to affect motor behaviour: response times, visuomotor coordination and driving skills are all impaired 1-2 hours after zolpidem administration, although the duration of these impairments is still under debate (Otmani *et al.*, 2008; Bocca *et al.*, 2011; Kleykamp *et al.*, 2012; Pilli *et al.*, 2013; Neylan *et al.*, 2020). Zolpidem also impairs sleep-dependent motor skill consolidation in humans, but does not seem to affect TMS-induced LTP-like plasticity (Morgan *et al.*, 2010; Lücke *et al.*, 2014). Whether zolpidem not only slows down response times but also impairs learning new motor skills is still unclear, as no studies to date have investigated the effect of zolpidem on motor learning shortly after drug administration.

6.1.6 Aim

I conducted a within-subject, double-blind, placebo-controlled study to investigate the effect of zolpidem on motor learning and brain chemistry. Young, healthy volunteers took part in two sessions when they received either a single dose of zolpidem (5mg) or placebo. Participants completed four sensorimotor tasks, which evaluated response times, motor sequence learning, visuomotor adaptation and tactile discrimination, and working memory tests. I also acquired transcranial magnetic stimulation (TMS) data at four time points during each session (before treatment administration, 1.6h, 2.35h, 3.35h after treatment administration) to evaluate cortical excitability and GABAergic inhibition. Before each set of TMS measurements, participants also filled in a mood questionnaire. **I hypothesised that zolpidem would significantly impair motor learning and improve tactile discrimination. I also hypothesised that zolpidem would change TMS metrics of GABAergic inhibition.**

6.2 METHODS

6.2.1 Participants

I recruited 20 healthy participants who took part in this study by giving their informed consent to all experimental procedures, as approved by the Central University Research Ethics Committee (Ethics reference: R74859/RE001). All participants met the following inclusion criteria: aged 18-35 years (mean age +/- SD 24.8 +/- 5.02 years, 10 males), right-handed as per the Edinburgh Inventory (Oldfield, 1971), no history of any psychiatric or neurological illness, not taking any medication, not musically trained (not more than Grade 6 on the Associated Board of the Royal Schools of Music) and TMS safety criteria.

6.2.2 Experimental design

I conducted a within-subject, double-blind, placebo-controlled study to investigate how increasing GABAergic inhibition via zolpidem, a $GABA_A$ receptor positive allosteric modulator, affected motor learning and brain chemistry. Participants attended two sessions at least one week apart, starting at the same time of day. The experimental design for the two sessions was identical (Fig. 6.1), except participants received a single dose of 5mg of zolpidem during one session and a placebo pill (sucrose/lactose) on the other. Both participant and experimenter were blinded to the treatment condition of each session, as the encapsulation and randomisation for this study were completed by Dr Margarita Chibalina at the NIHR Oxford Health Biomedical Research Centre.

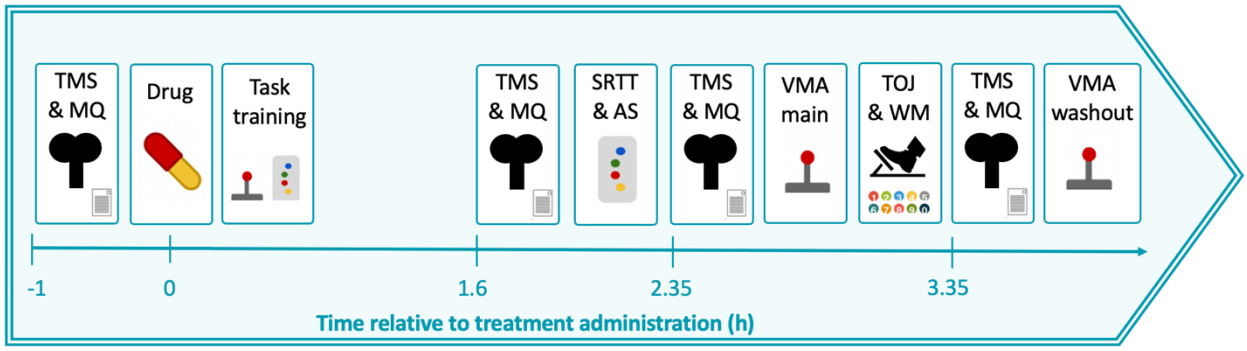


Fig. 6.1. **Timeline of the testing session.** After the TMS set-up and first set of TMS measurements, the participant received the treatment (zolpidem or placebo) and then were familiarised with the tasks (action selection [AS], serial reaction time task [SRTT], temporal-order judgement and visuomotor adaptation [VMA]), which lasted approximately 15 minutes. At 1.6h post-treatment administration (PA), I collected another set of TMS measurements, after which participants completed the AS and the SRTT tasks. Then wIe collected another set of TMS measurements (2.35h PA), after which participants completed the VMA main task, the TOJ task and two working memory tests: the digit span and the auditory verbal learning test. I then collected the last set of TMS measurements (3.35h PA), after which participants completed the VMA washout task. Before the TMS measurements at each time point (baseline, 1.6h, 2.35h and 3.35h), we also asked the participants to fill in a mood questionnaire (MQ).

At the start of each session, participants completed a TMS safety screening form and a mood questionnaire, specifically the Bond Lader Visual Analog Scale (BLVAS). I then measured the resting and active motor thresholds and collected baseline TMS measurements, as detailed below. The participants then received the treatment (5 mg zolpidem, placebo) and completed the task familiarisation training. I then waited for 1hour and 40 minutes for zolpidem to be absorbed and reach peak plasma concentration, similar to previous pharmaco-TMS studies using zolpidem (Di Lazzaro *et al.*, 2006; Teo *et al.*, 2009). TMS metrics and mood questionnaires were then collected at 3 time points after treatment administration: 1.6h, 2.35h and 3.35h. Between the first two, participants executed the action selection (AS) task and the serial reaction time task (SRTT), and then, between the last two, they executed the visuomotor adaptation (VMA) main task, the temporal-order judgement (TOJ) task and the two working memory tests (digit span and auditory verbal learning test). Lastly, participants completed the VMA washout task.

6.2.3 Sensorimotor tasks

6.2.3.1 Action Selection (AS) task

As in Chapter 3, I used the AS task as a non-learning motor task to evaluate the participants' execute and select response times (O'Shea *et al.*, 2007). Participants were familiarised with a 1-minute version of the task immediately after treatment administration. They completed the full-length AS task (6.5 minutes) at approximately 2 hours post-treatment administration.

Participants were instructed to use their right index finger to press button 1 (o) and their right middle finger to press button 2 (p) on a laptop (Dell Latitude E5540). The AS task was composed of two types of blocks: Execute blocks (EBs) and Select blocks (SBs), each containing 34 trials. During execute blocks, participants were instructed to press the button listed in the instruction screen for each cue they saw (Fig. 6.2a). During select blocks, they were told to press button 1 if they saw a small square or a big circle, and press button 2 if they saw a small circle or a large square (Fig. 6.2b). In total, the AS task had 4 blocks (2 EBs and 2 SBs), which were completed in random order.

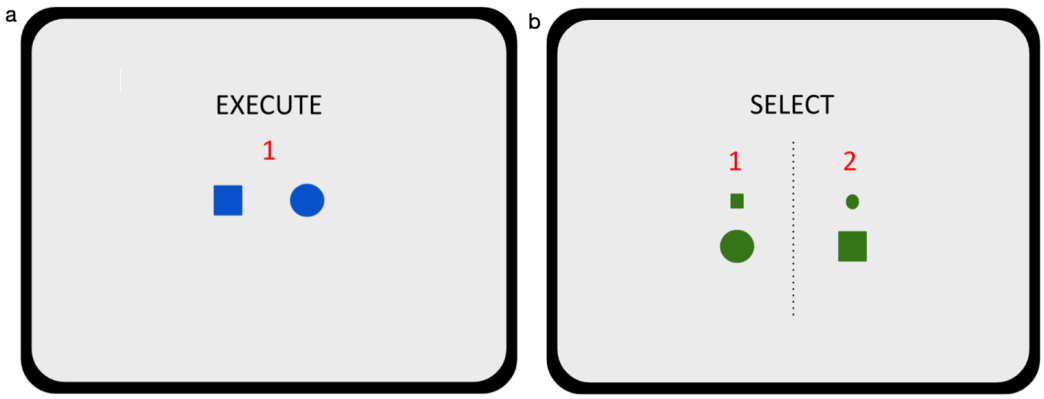


Fig. 6.2**. AS instructions and block order**. a) Sample EB instructions; b) Sample SB instructions

6.2.3.1.1 AS data analysis

I calculated response times (RTs) for execute and select blocks as the time between stimulus presentation and the button press. I excluded trials with incorrect button presses, pre-emptive button presses (RT < 50ms), long RTs (RTs > 3s) and outliers (± 2.7 SD from the mean of each block type). The task accuracy for all participants was over 70%. I then calculated the median RT for each block type for each participant: the median RT for execute blocks was considered the participants' response time, and the difference between the median RT of select and execute blocks (SB – EB) was considered the participants' action-selection time.

6.2.3.2 Serial Reaction Time Task (SRTT)

To assess motor sequence learning, I used a similar SRTT task as in Chapter 3. Participants used their right index, middle, ring, and little fingers to press buttons 1-4 on a 4-button box in response to a visual cue, as quickly and accurately as possible. Participants were instructed to not press a button before the visual cue appeared on the screen.

At the beginning of each block, participants would see whether the following block was a random or a sequence block and then a screen with four dashes (Fig. 6.3a). One of the dashes would then be replaced by a circle (Fig. 6.3b), which represented the visual stimulus for the participant to press the corresponding button. The SRTT was composed of 12 blocks in total, 2 random blocks flanking 10 sequence blocks, each with 48 self-paced trials. The block type was associated with different colours for the stimuli: black stimuli for random blocks and blue stimuli for sequence blocks. During random blocks, the order of the visual cues was random, so the participants could not anticipate which button would come next. During each sequence block in a session, the same 12-button sequence

was repeated four times. As participants repeat the sequence and learn which button comes next, their RTs decrease, indicating better motor learning (Robertson, 2007). Two different sequences were used for the two sessions.

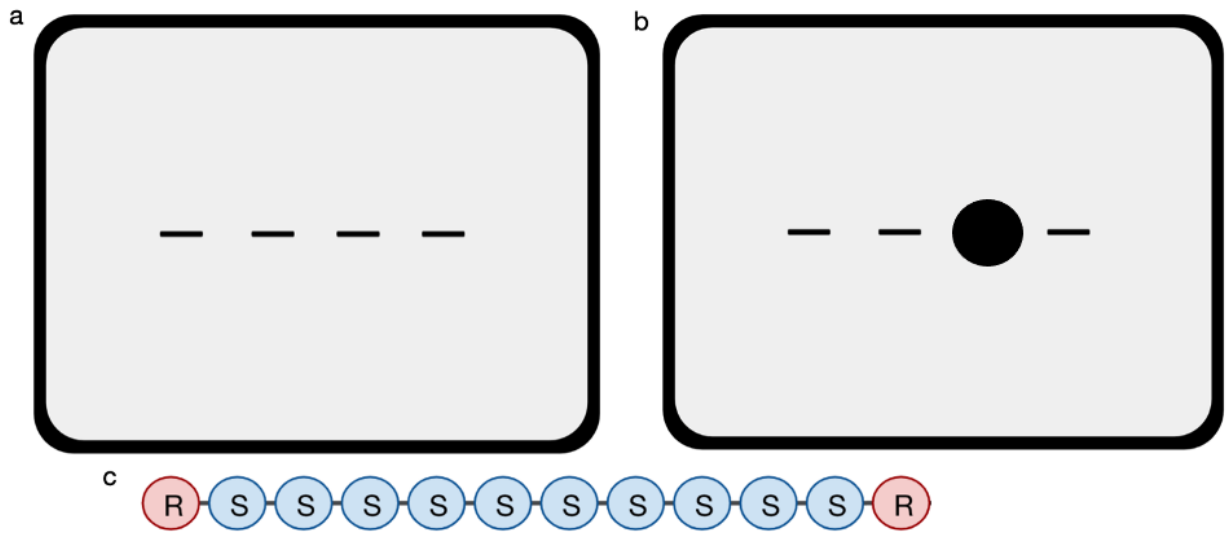


Fig. 6.3. **Serial reaction time task (SRTT)**. a) Rest screen; b) Sample cue for a random block; c) Block order

6.2.3.2.1 SRTT data analysis

I calculated response times (RTs) for each block as the time between stimulus presentation and the button press. I excluded trials with incorrect button presses, pre-emptive button presses (RT < 50ms), long RTs (RT > 1.5ms) and outliers (± 2.7 SD from the mean of each block). I then calculated the median RT for each block and normalised it by dividing it by the median RT of the first random block (R1). I also calculated a learning gradient for each session by fitting a linear regression model to the data in sequence blocks 1 to 10. One participant was excluded from further analysis because their learning gradient on one session was classified as an outlier on the Grubbs test.

6.2.3.3 Visuomotor Adaptation (VMA) tasks

Similar to Chapter 3, I asked participants to complete a visuomotor adaptation task to measure the participants' ability to adapt to perturbations of visuomotor mapping and retain this motor skill (Galea *et al.*, 2011). Before treatment administration, participants trained on a shorter version of the task (5 minutes), which did not include any perturbation. They then completed the full-length main task (20 minutes) at approximately 2.6 hours post-treatment administration. At approximately 3.6h post-treatment administration, participants also completed a washout task (12 minutes), which did not include any perturbation; the washout task was used to measure motor skill retention and reduce any potential carry-over effects between sessions.

Participants used their right hand to move a joystick (Logitech Attack 3), which controlled a cursor on the computer screen (Dell Latitude E5540). For both VMA tasks, the participant's right hand was covered by a shielding box to prevent visual feedback of the hand's movements.

6.2.3.3.1 VMA main task

At the beginning of each trial, the joystick would be in the neutral position, placing the red cursor in the centre of the screen (Fig.M4a). For each trial, participants were instructed to make quick shooting movements toward the yellow target as accurately as possible, but without adjusting their trajectory during the trial.

The task was composed of two types of blocks: feedback-on blocks (FB-ON) and feedback-off blocks (FB-OFF). During FB-ON blocks, there was a 30° counterclockwise perturbation between the participant's hand movement and the trajectory of the cursor on the screen,

so participants would see a 30°-deviated trajectory (Fig. 6.4b). This was unknown to the participants, who then had to adjust their hand movements to compensate for the perturbation. During FB-OFF, the cursor disappeared as soon as the participants moved the joystick. The lack of visual feedback allowed us to measure if participants retained the 30° perturbation to visuomotor mapping. In total, the task had 10 blocks, with alternating FB-ON and FB-OFF blocks. Each block contained five epochs; each epoch contained 8 consecutive trials, one for each defined target (Fig. 6.4a).

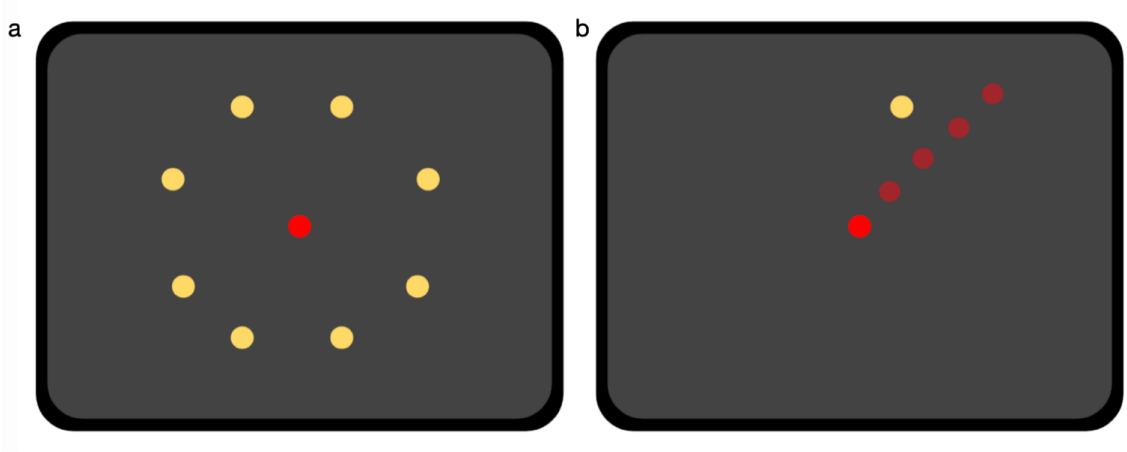


Fig. 6.4**. Visuomotor adaptation task (VMA).** a) Target circle: 8 potential targets (yellow) and cursor (red); b) Cursor trajectory in FB-ON blocks

6.2.3.3.2 VMA washout task

The VMA washout task was similar to the main task, except no perturbation was present in the FB-ON blocks. The washout task was also shorter (6 blocks in total) and started with a FB-OFF block. The number of epochs within a block and the number of trials within an epoch were identical to the VMA main task.

6.2.3.3.3 VMA data analysis

For each trial on both tasks, I calculated the angular error as the angle between the target location and the point where the cursor crossed the imaginary target circle. On the trials where the cursor trajectory did not reach the imaginary target circle, the angular error was calculated at the point where the cursor crossed a circle with a radius half of that of the target circle. The trial was excluded if any of the following three conditions applied: cursor trajectory not reaching the half-radius point, angular error over 67.5^{o} or the joystick not being in the neutral position at the start of the trial. I then calculated the mean angular error for each epoch and each participant and used it in the statistical analysis.

For some participants, there was an error in saving the data from the last block: 3 participants with 4 missing epochs in one of the sessions. For two other participants, the main task ended suddenly during the second to last FB-OFF block due to computer malfunction, so I had 2 participants with 12 missing epochs in one session. Even if these 2 participants completed the remaining FB-ON and FB-OFF, I only included the data up to the interruption in the VMA main task analysis. I also excluded one of these participants from the washout task analysis for completing a longer main task than the rest of the participants.

6.2.3.4 Temporal-Order Judgement (TOJ) task

To assess the participants' tactile discrimination, I used a temporal-order judgement task, as previously described (Zampini *et al.*, 2005). Participants were presented with two consecutive vibrotactile stimuli to their right index and middle fingers. These stimuli came at 7 different interstimulus intervals (300ms, 150ms, 100ms, 75ms, 50ms, 30ms, 15ms);

in half of the trials, the first stimulus was delivered to the right index finger and the other half to the right middle finger. Participants were instructed to decide which finger received the first stimulus and press the corresponding pedal with their foot: the left pedal when their index finger received the first stimulus and the right pedal when their middle finger received the first stimulus. Participants had 2 seconds to respond, after which the current trial answer was void and the next trial began. Each of the 14 interstimulus intervals (7 ISIs x 2 first-finger conditions) was presented in 12 trials in random order.

During the entire task, the participants listened to pink noise through headphones, covering any potential noise coming from the tactile stimulators, and their right hand was obscured with a shielding box.

6.2.3.4.1 TOJ data analysis

I first excluded any trials where no pedal or both pedals were pressed. Then I calculated the proportion of right-first responses for each interstimulus interval and fitted a logistic function to this data using MATLAB R2021b (MathWorks). Data from one participant could not be collected due to equipment malfunction. Data from two participants were excluded due to poor goodness-of-fit to the logistic function ($R^2 < 0.4$) in one or two of the sessions, as previously described (Azañón *et al.*, 2015), and data from another participant was excluded because their R^2 was an outlier on the Grubbs test.

I calculated the just-noticeable difference (JND), as half of the difference between the two ISIs where participants chose right-first in 75% and 25% of the trials. A smaller JND, therefore, indicates better tactile discrimination.

6.2.4 Transcranial Magnetic Stimulation (TMS)

6.2.4.1 TMS data collection

The entire TMS dataset was collected using a monophasic DuoMAG machine connected to a 70mm figure-of-eight coil (BrainBox, Cardiff) to stimulate the participants' left primary motor cortex. For the whole duration of TMS data collection, the participants were comfortably seated in a chair, with their right arm resting on the desk in front of them, and were instructed to keep their eyes open.

First, I identified the motor hotspot, the optimal scalp position of elicit MEPs in the right first dorsal interosseous (FDI) muscle, and marked it on the participants' scalp to aid coil positioning. I held the TMS coil at a 45^0 to the midsagittal line with the handle pointing posteriorly.

Surface electromyography was recorded using Kendall disposable neonatal ECG electrodes (Cardinal Health) from the right FDI muscle using a belly-tendon montage with a ground electrode over the ulnar styloid process. The data was sampled at 5kHz, amplified, filtered (10Hz – 1kHz) and recorded using a Digitimer D440 4-Channel Isolated Amplifier (Digitimer), a CED micro1401 A/D converter and a Signal software version 7.06 (Cambridge Electronic Design). A Digitimer Mains Noise Eliminator (Digitimer) was used to reduce 50Hz electrical noise.

I did not record TMS measurements from two participants, one due to failure to find reliable MEPs at a TMS intensity of 75% of maximum stimulator output and the other due to reports of sharp pain to TMS pulses delivered at 65% of maximum stimulator output.

These two participants completed both sessions as described above, only without the acquisition of TMS data.

6.2.4.2 Motor thresholds

I determined the resting 1mV motor threshold (rMT) and the active motor threshold (aMT) before treatment administration (baseline), during the TMS set-up. The rMT was defined as the lowest stimulation intensity that elicited an MEP with a peak-to-peak amplitude of 1mV in at least 5 out of 10 consecutive trials in the FDI muscle at rest. The aMT was defined as the lowest stimulation intensity necessary to elicit an MEP with a peak-to-peak amplitude of over 0.2mV in at least 5 out of 10 consecutive trials when the participants were contracting their right FDI at approximately 30% of their maximum contraction.

The resting motor threshold can be used as a measure of cortical excitability, with higher stimulation intensity being associated with a lower cortical excitability (Rossini *et al.*, 1994; Abbruzzese & Trompetto, 2002). I recorded the rMT at 1.6h, 2.35h and 3.35h after treatment administration, to investigate the effect of zolpidem on cortical excitability.

6.2.4.3 Paired-pulse TMS protocols

I collected two types of paired-pulse TMS protocols: short-interval intracortical inhibition (SICI) at two interstimulus intervals (2.5ms and 1ms), and long-interval intracortical inhibition (LICI) at two interstimulus intervals (150ms and 100ms). These paired-pulse protocols can be used as TMS metrics of GABA-mediated inhibition; specifically, SICI 2.5ms is thought to reflect $GABA_A$ synaptic activity, while SICI 1ms has been hypothesised to reflect $GABA_A$ extrasynaptic activity and LICI 150ms that of $GABA_B$-receptor activity

(Ziemann *et al.*, 1996*b*; Fisher *et al.*, 2002; Ilić *et al.*, 2002; Roshan *et al.*, 2003; Ziemann, 2004; McDonnell *et al.*, 2006; Ni *et al.*, 2007; Müller-Dahlhaus *et al.*, 2008; Stagg *et al.*, 2011*b*). For each SICI measurement, the test stimulus was set at the value for rMT and the conditioning stimulus was set at 70% aMT. For each LICI measurement, both the test and the conditioning stimuli were set at the value for rMT.

During each paired-pulse protocol, I first identified the new rMT for the time point and then I recorded 12 trials of single test pulses at the new rMT and 12 pulses of each interstimulus interval delivered in a pseudorandomised order.

6.2.4.4 MEP data analysis

I excluded all trials with precontraction in the right FDI muscle (EMG amplitude > 0.15mV in the 50ms preceding the test pulse). I then calculated the peak-to-peak MEP amplitude for each trial and excluded all trials with an MEP amplitude > 3mV and single test pulses with an MEP amplitude < 0.15mV. Paired-pulse trials with an MEP amplitude < 0.1mV where the preceding single test pulse had had an MEP amplitude < 0.15mV were also excluded. I then calculated the mean MEP value for each ISI and excluded outliers (> 2SD from the mean or outliers at the Grubbs test). I calculated the (mean paired-pulse MEP)/(mean single-pulse MEP) ratio for each ISI so that smaller values would indicate more GABAergic inhibition. These values were used in all further TMS data analyses.

6.2.5 Working memory tests

Working memory is involved in learning new motor skills (Seidler *et al.*, 2012). I investigated the effect of zolpidem on working memory to determine whether any potential effects of zolpidem on motor learning might arise from effects on working

memory. I asked participants to complete two working memory tests, the Digit Span and the Rey Auditory Verbal Learning Test (AVLT), at approximately 3.2h after treatment administration.

6.2.5.1 Digit Span

Participants completed both the forwards and backwards versions of the digit span (Richardson, 2007; Woods *et al.*, 2011). For both versions, I began with sequences of 2 digits that we read to the participant and asked them to recall them. For the forwards version, I asked participants to recall the sequence in the same order it was said, and for the backwards version, we asked them to recall the sequence in the reverse order. For each sequence length, I read two sequences and progressed to the next sequence length if the participant correctly recalled at least one of the two sequences. For each version, I recorded the highest sequence length at which the participant correctly recalled at least one sequence. The maximum sequence length was 10 digits. Longer sequence lengths indicate better performance on the digit span.

6.2.5.2 Rey Auditory Verbal Learning Test (AVLT)

The AVLT consists of a list of 15 words which the researcher repeated to the participants 5 times (Rey, 1958; Spreen & Strauss, 1998; Van der Elst *et al.*, 2005). After each repeat, the participant was asked to recall as many of the 15 words as they could in no particular order. After the 5th repeat, the participant was presented with a second list of 15 words and again was asked to recall as many words as they could from the second list. Then, the participant was asked to recall the words from the first list again (immediate list recall). After the 3h PA TMS data collection and the VMA washout task, the participant was asked

to recall the words from the first list again (delayed list recall). The two lists were different between the two sessions.

In each session, I recorded the number of words correctly recalled after each repeat (R1-R5) and for the immediate and delayed list recalls (maximum 15 each time). More recalled words indicate better performance on the AVLT.

6.2.6 Mood questionnaires

To quantify any zolpidem-related changes in mood, I asked participants to fill in the Bond-Lader Visual Analogue scale (BLVAS) four times during each session before each TMS acquisition: before (baseline), 1.6h, 2.35h and 3.35h post-treatment administration. As described in chapter 4, the BLVAS has 16 scales, each 100mm long with the 2 opposite sides of the spectrum at each end, e.g. alert – drowsy, calm – excited (Bond & Lader, 1974). At each time point, participants were instructed to make a mark on each scale for how they felt at that specific moment. The scores on the 16 scales were grouped into 3 categories: alertness (alert, strong, clear-headed, well-coordinated, energetic, quick-witted, attentive, proficient, interested), contentedness (contented, tranquil, happy, social, friendly) and calmness (calm, relaxed) (Bond & Lader, 1974). The mean value for each category was calculated at each time point, with lower values indicating stronger feelings on that respective scale. Data from one time point (3.35h) was not recorded from one participant.

6.2.7 Statistical analysis

All behavioural and TMS data were analysed using MATLAB (v. R2021b, MathWorks) and Prism (v.9.3.0, GraphPad Software). Normality of data distribution was investigated using the D'Agostino & Pearson test. Analysis was performed using repeated-measures ANOVA or paired t-tests with significance levels of α = 0.05 to investigate the within-subject effects of zolpidem compared to placebo. Where the normality criterion was not fulfilled, I performed non-parametric tests. For the datasets with missing values, where I could not perform repeated-measures ANOVA, I used mixed-effects models (restricted maximum likelihood) with within-subject fixed effects of treatment and time, as specified in the results section. All graphs show mean ± SD or individual data points unless otherwise indicated.

6.3 RESULTS

6.3.1 Zolpidem significantly increased response times and actions-selection times

First, I investigated whether zolpidem affected motor performance. I used two Wilcoxon matched-pairs signed-rank tests to determine the effect of zolpidem on participants' response time to visual stimuli (execute RTs) and action-selection times (select – execute RTs). I found that zolpidem significantly increased both response times on execute blocks ($T(19) = -25$, $p = 0.0017$; Fig. 6.5A) and action-selection time ($T(19) = -34$, $p = 0.0064$; Fig. 6.5B), indicating that zolpidem significantly impaired motor performance. To confirm that the effect on action-selection times was not a result of slower response times with zolpidem, I compared the median RTs on execute and select blocks between treatment conditions. Using a two-way repeated measure ANOVA with within-subject factors of treatment (zolpidem, placebo) and difficulty (execute RTs, select RTs), I found a significant effect of difficulty ($F(1, 19) = 247.8$, $p < 0.0001$), a significant effect of treatment ($F(1, 19) = 14.94$, $p = 0.001$) and a significant difficulty x treatment interaction ($F(1,19) = 7.649$, $p = 0.012$); indicating that zolpidem slowed down both response times and action-selection times.

To determine a potential session order effect, I used two Wilcoxon matched-pairs signed-rank tests on participants' response time to visual stimuli (execute RTs) and action-selection times (select – execute RTs) between session 1 and session 2, regardless of treatment group. I found no significant effect of session (execute RTs: $T(19) = -93$, $p = 0.674$; action-selection times: $T(19) = 98$, $p = 0.812$).

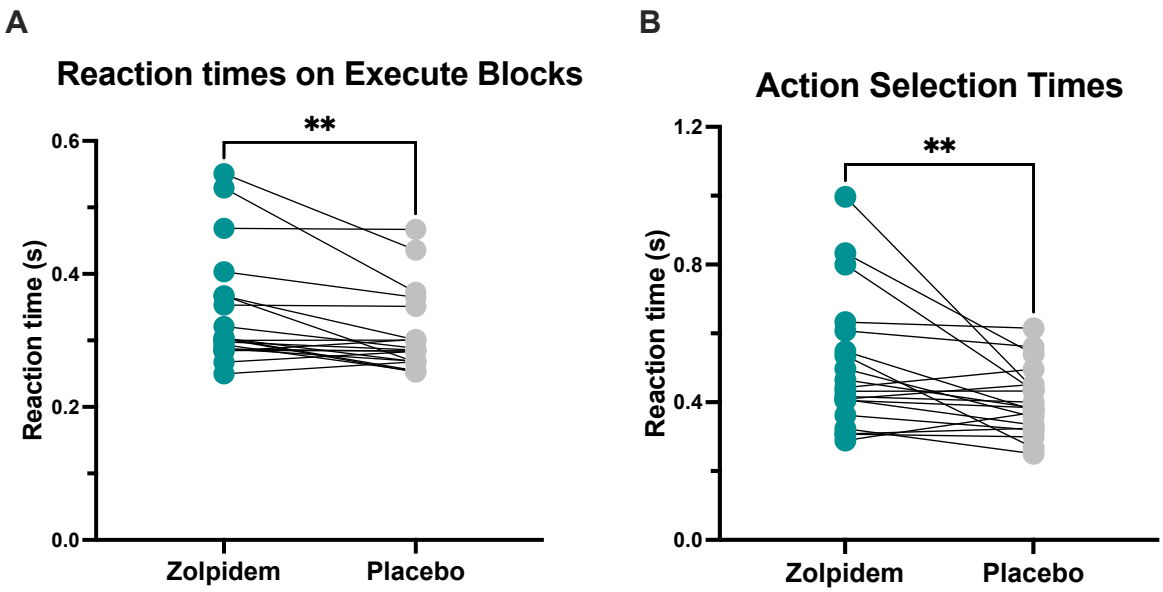


Fig. 6.5**. Behaviour on Action Selection Task**. Both response times on execute blocks (A) and action selection times (B) were increased with zolpidem compared to placebo. **p<0.01

6.3.2 Zolpidem significantly impaired performance on the SRTT

I next investigated the effect of zolpidem on motor sequence learning by comparing the participants' performance on the SRTT between treatment conditions. The mean response times of the first and second random block were significantly lower with zolpidem than placebo (Wilcoxon matched-pairs signed-rank test: $T(18) = -35$, $p = 0.014$). I compared the median RTs during sequence blocks between treatment conditions using a two-way repeated-measures ANOVA with within-subject factors of treatment (zolpidem, placebo) and learning (S1,S2,…S10) and found a significant effect of treatment ($F(1, 18) = 12.79$, $p = 0.0022$), a significant effect of learning ($F(2.831, 50.95) = 6.822$, $p = 0.0007$), but no significant treatment x learning interaction ($F(4.963, 89.33) = 1.474$, $p = 0.207$; Fig. 6.6A). When normalised median RTs were used (for each sequence block, median RT divided by median RT on random block 1) in the same analysis, I found a significant effect of learning ($F(2.288, 41.18) = 7.351$, $p = 0.0012$), but no significant effect of treatment ($F(1, 18) = 2.560$, $p = 0.127$) or learning x treatment interaction ($F(4.767, 85.81) = 1.467$, $p = 0.2114$).

I also calculated the skill that participants acquired during motor sequence learning, by subtracting the response times at the end of sequence learning (sequence block 10) from the last random responses (R2 – S10). Using a paired t-test, I compared the acquired skill between treatment conditions and found no significant difference between zolpidem and placebo ($t(18) = 0.862$, $p = 0.400$).

I then investigated whether there was an effect of session on RTs during sequence blocks by using a two-way repeated-measures ANOVA with within-subject factors of session (session 1, session 2) and learning (S1, S2,…S10). I found a significant effect of learning

(*F(2.831, 50.95)* = 6.822, *p* = 0.0007) and a significant learning x session interaction (*F(5.049, 90.88)* = 2.340, *p* = 0.048), but no significant effect of session (*F(1, 18)* = 0.0036, *p* = 0.953).

To control for this effect of session when investigating the effect of zolpidem on learning, I used a three-way ANOVA with within-subject factors of treatment (zolpidem, placebo) and learning (S1, …S10) and between-subject factor of order (zolpidem on first session, placebo on first session). I found a significant effect of learning (*F(1.650, 28.05)* = 6.137, *p* = 0.009) and a significant learning x treatment x order interaction (*F(9, 153)* = 3.001, *p* = 0.0025). Nothing else was statistically significant (main effect of order (*F(1, 17)* = 4.325, *p* = 0.053); main effect of treatment (*F(1, 17)* = 2.744, *p* = 0.116); learning x order interaction (*F(9, 153)* = 0.370, *p* = 0.948); learning x treatment interaction (*F(4.497, 76.45)* = 0.821, *p* = 0.528); order x treatment interaction (*F(1, 17)* = 0.424), *p* = 0.524). To further dissect this result, I investigated whether there was any difference in RTs during learning between treatment conditions on each session. For each session (session 1, session 2), I used a two-way ANOVA with within-subject factor of learning (S1, …S10) and between-subject factor of treatment (zolpidem, placebo). On the first session, I found a significant effect of learning (*F(9, 153)* = 6.154, *p* < 0.0001), but no significant effect of treatment (*F(9, 153)* = 1.047, *p* = 0.321) and no significant treatment x learning interaction (*F(9, 153)* = 0.537, *p* = 0.846) (Fig. 6.6B). On the second session, I found a significant effect of treatment (*F(1, 17)* = 11.78, *p* = 0.0032), but no significant effect of learning (*F(1.249, 21.22)* = 3.010, *p* = 0.09) and no significant treatment x learning interaction (*F(9, 153)* = 0.529, *p* = 0.852) (Fig. 6.6C). I then further investigated whether learning was present in any of the 4 separate groups (zolpidem session 1, zolpidem session 2, placebo session 1,

placebo session 2) using a one-way repeated-measures ANOVA with within-subject factor of learning (S1, …, S10). For both placebo sessions, I found a significant effect of learning (session 1: *F(2.260, 20.34)* = 4.076, *p* = 0.029; session 2: *F(2.386, 19.09)* = 3.846, *p* = 0.033). However, on both zolpidem sessions, there was no significant effect of learning (session 1: *F(2.575, 20.60)* = 2.584, *p* = 0.0882; session 2: *F(3.652, 32.86)* = 1.122, *p* = 0.360). This would indicate that zolpidem significantly impairs motor sequence learning.

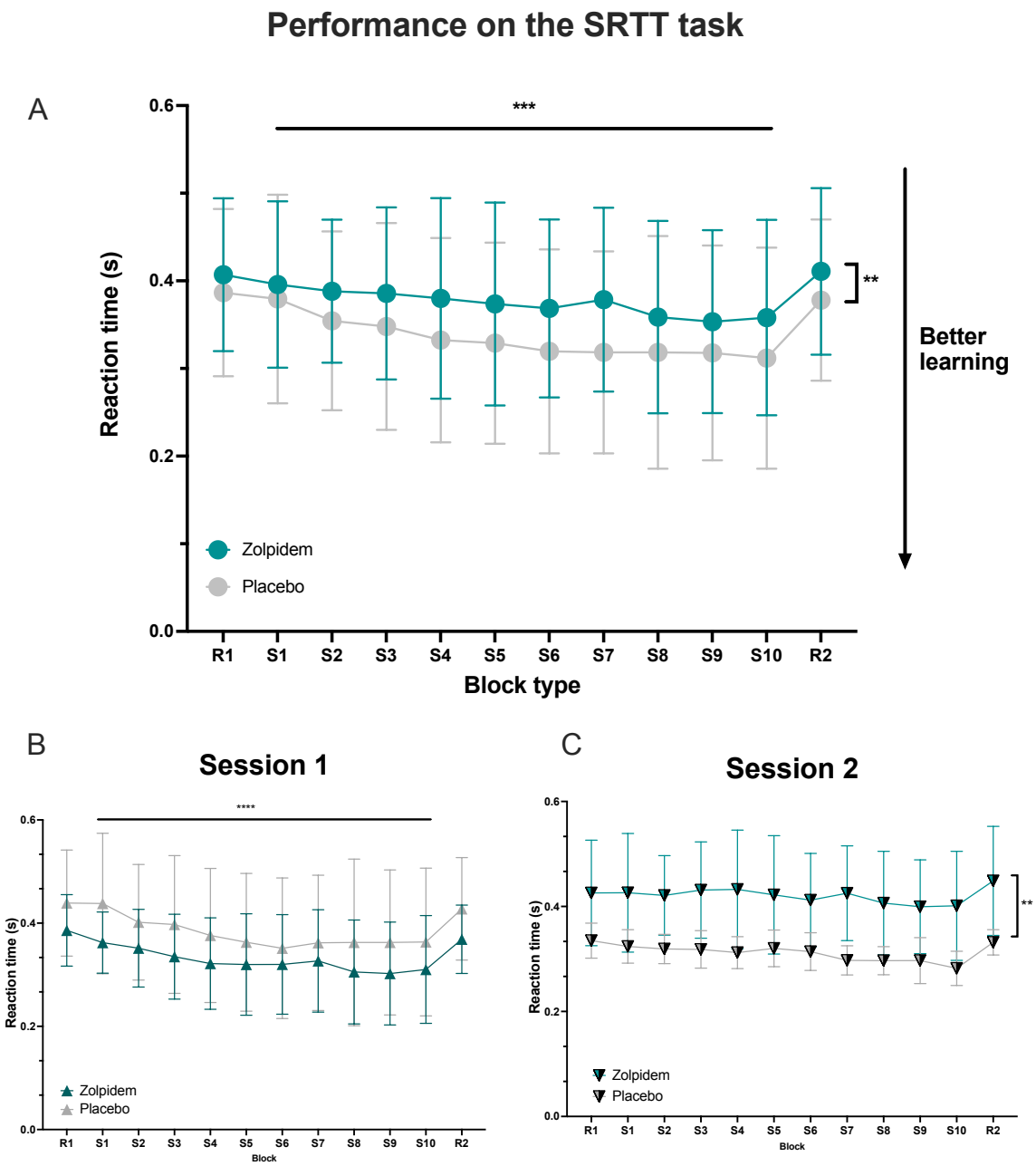


Fig. 6.6**. Behaviour on the Serial Reaction Time Task.** Response times during sequence learning were significantly slower after zolpidem administration compared to placebo (A). Learning RTs between treatment conditions on session 1 (B) and session 2 (C)

6.3.3 Zolpidem significantly impaired retention of visuomotor adaptation skills

I then investigated whether zolpidem affected visuomotor adaptation and retention. As described above, I measured the mean angular error between the target presented on the screen and the participants' trajectory during both feedback-on and feedback-off blocks. Due to technical issues described in Methods, the data from the final epochs of the main task in 5 of the participants were missing. Therefore, for the main task, the mean angular error for the 5 epochs in each block was used in a mixed-effects model (restricted maximum likelihood) with within-subject fixed effects of treatment (zolpidem, placebo), block (B1, … B5) and feedback (FB-ON, FB-OFF) (Fig. 6.7A). For the washout task, the mean angular error for each block was used in a three-way repeated-measures ANOVA with within-subject factors of treatment (zolpidem, placebo), block (B1, B2, B3) and feedback (FB-ON, FB-OFF) (Fig. 6.7B).

For the main task, I found a significant effect of block ($F(2.668, 50.69) = 10.38$, $p < 0.0001$) and feedback ($F(0.354, 6.722) = 2830$, $p < 0.0001$) and a significant block x feedback interaction ($F(4, 76) = 3.177$, $p = 0.018$), but no significant effect of treatment ($F(0.718, 13.64) = 2.706$, $p = 0.127$), no significant block x treatment ($F(4, 76) = 0.260$, $p = 0.903$), feedback x treatment ($F(0.786, 14.94) = 0.129$, $p = 0.661$) or block x treatment x feedback interactions ($F(1.909, 34.36) = 0.542$, $p = 0.578$). This would indicate that while participants learnt to adapt their movements to compensate for the perturbation, zolpidem did not significantly affect this process.

For the washout task, I found a significant effect of block ($F(1.312, 23.62) = 38.37$, $p < 0.0001$) and feedback ($F(0.550, 9.893) = 19.35$, $p = 0.004$) and a significant block x

treatment interaction (*F(2, 36)* = 4.354, *p* = 0.020), but no significant effect of treatment (*F(0.755, 13.59)* = 0.149, *p* = 0.633), no significant feedback x treatment (*F(0.741, 13.34)* = 0.903, *p* = 0.327) or block x treatment x feedback interactions (*F(1.375, 24.74)* = 0.707, *p* = 0.452). This would indicate that, with no perturbation present, participants returned to their default sensorimotor mapping; this de-adaptation process was significantly affected by zolpidem.

I then investigated whether these findings were affected by potential carry-over effects between sessions. For the main task, I used a mixed-effects model (restricted maximum likelihood) with within-subject fixed effects of session (session 1, session 2), block (B1, .. B5) and feedback (FB-ON, FB-OFF). For the washout task, the mean angular error for each block was used in a three-way repeated-measures ANOVA with within-subject factors of session (session 1, session 2), block (B1, B2, B3) and feedback (FB-ON, FB-OFF). I found no significant effect of session for either task (main task: *F(0.716, 13.61)* = 0.057, *p* = 0.728; washout: *F(0.785, 14.91)* = 0.249, *p* = 0.567) and no significant interactions (main task: block x session: *F(4, 76)* = 1.329, *p* = 0.267, feedback x session *F(0.768, 14.58)* = 3.699, *p* = 0.081, block x feedback x session *F(2.053, 36.95)* = 0.031 *p* = 0.972; washout: block x session *F(2, 38)* = 0.785, *p* = 0.463, feedback x session *F(0.743, 14.12)* = 0.0428, *p* = 0.764, block x feedback x session *F(1.379, 24.14)* = 2.012), *p* = 0.165). This indicates that there were no significant carry-over effects for this task.

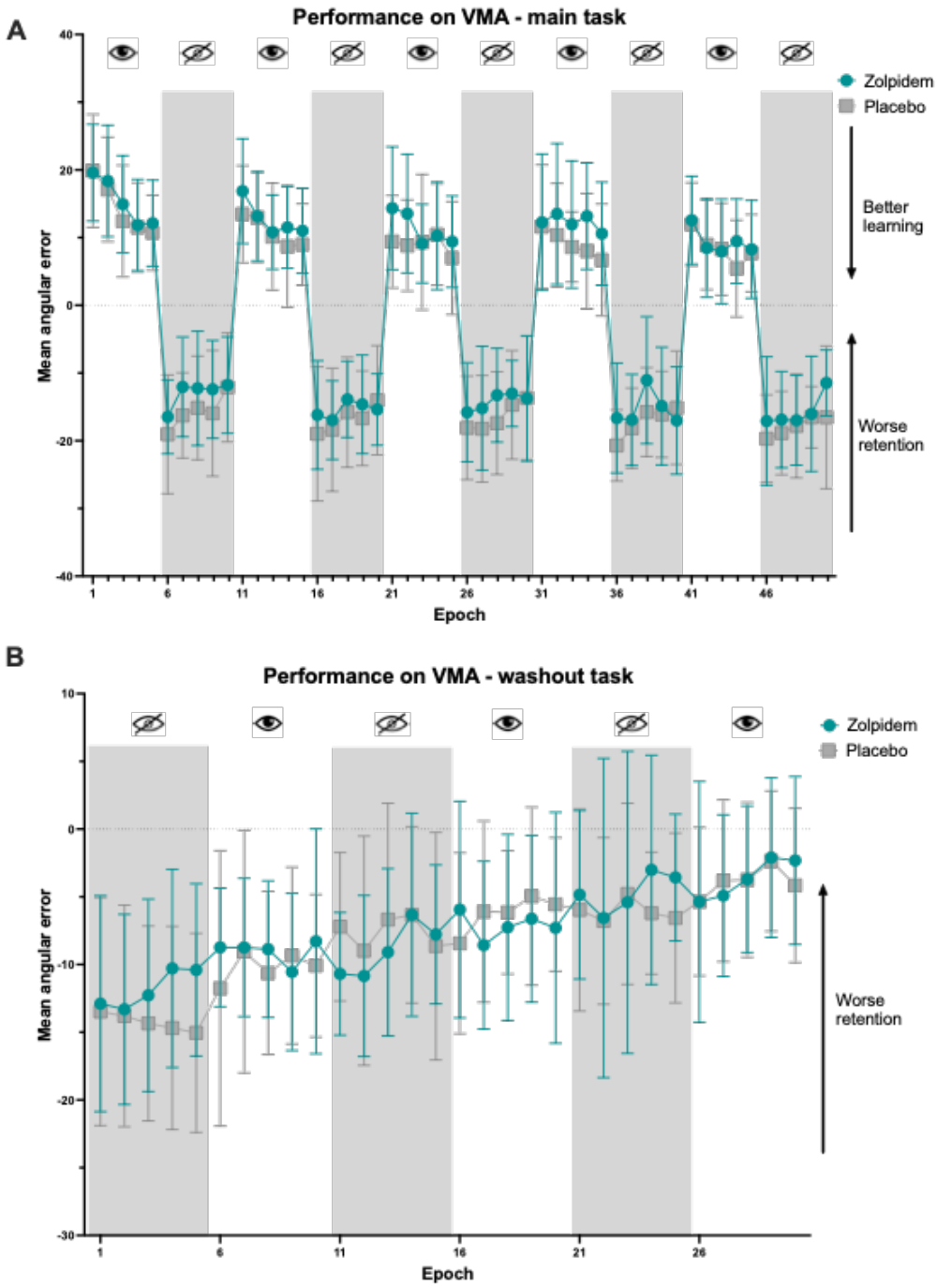


Fig. 6.7**. Behaviour on the visuomotor adaptation tasks: (A) main task, (B) washout task.** No significant difference in mean angular errors during feedback-on blocks (white background) and feedback-off blocks (grey background) between zolpidem and placebo session were detected on during the main task (A). Significantly worse retention with zolpidem was detected on the washout task (B)

6.3.4 Zolpidem significantly impaired touch perception

To assess the effect of zolpidem on tactile perception, I compared the participants' performance on the temporal-order judgement (TOJ) task between treatment conditions. I fitted a logistic function to the participants' data, as described above, and calculated the just-noticeable difference (JND) on the zolpidem and placebo sessions. I used a paired t-test to compare the goodness-of-fit between treatment conditions and found a significantly decreased goodness-of-fit on zolpidem sessions compared to placebo ($t(15) = 2.906$, $p = 0.011$; Fig. 6.8A). I then used a paired t-test to compare the JND between treatment conditions and found that it was significantly increased during zolpidem sessions compared to placebo ($t(15) = 2.579$, $p = 0.021$; Fig. 6.8B). This indicates that zolpidem significantly impaired tactile discrimination. To determine whether there was an effect of session, I used two Wilcoxon matched-pairs signed-rank tests to compare the goodness-of-fit and JND between sessions and found no significant differences between sessions (goodness-of-fit: $T(15) = 54$, $p = 0.495$; JND: $T(15) = 44$, $p = 0.231$).

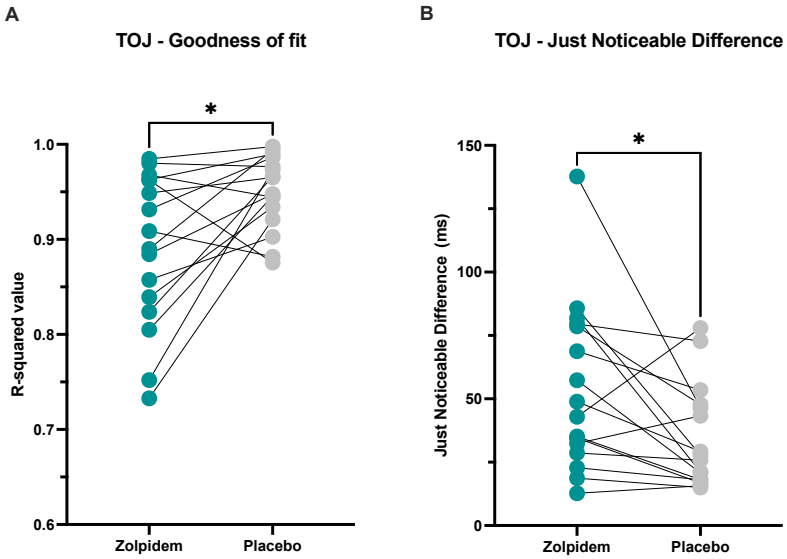


Fig. 6.8**. Performance on the TOJ task**. Zolpidem significantly decreased the goodness-of-fit (A) and increased the JND (B) compared to placebo. * $p < 0.05$

6.3.5 Zolpidem did not significantly change TMS metrics of cortical excitability and GABAergic inhibition

To investigate the effect of zolpidem on cortical excitability, I recorded the participants' resting motor threshold as percentage of maximum stimulator output at each time point: before treatment administration (baseline), 1.6h, 2.35h and 3.35h post-treatment administration. I then compared these values between treatment conditions using a mixed-effects model (restricted maximum likelihood) with within-subject effects of treatment (zolpidem, placebo) and time (baseline, 1.6h, 2.35h, 3.35h). I found no significant effect of treatment ($F(1,17) = 0.499$, $p = 0.490$) or time ($F(2.348, 39.91) = 2.793$, $p = 0.065$) and no significant time x treatment interaction ($F(2.530, 41.32) = 0.181$, $p = 0.880$), which indicates zolpidem did not significantly affect cortical excitability (Fig. 6.9A).

I then studied the effect of zolpidem on TMS metrics of $GABA_A$-mediated inhibition: SICI 2.5ms and SICI 1ms. For each SICI interstimulus interval and each time point, I calculated the ratio between the mean amplitude of the paired-pulse and the single-pulse MEP, so that a smaller value represents more inhibition. I then compared these values between treatment conditions using a two-way repeated measures ANOVA with within-subject factors of treatment (zolpidem, placebo) and time (baseline, 1.6h, 2.35h, 3.35h). I found no significant effect of treatment (SICI 2.5ms: $F(1,17) = 1.078$, $p = 0.314$; SICI 1ms: $F(1,17) = 2.688$, $p = 0.1195$) or time (SICI 2.5ms: $F(2.175, 36.97) = 1.252$, $p = 0.300$; SICI 1ms:, $F(2.715, 46.15) = 0.627$, $p = 0.587$) and no significant time x treatment interaction (SICI 2.5ms: $F(2.635, 44.80) = 1.554$, $p = 0.217$; SICI 1ms:, $F(2.359, 40.11) = 0.241$, $p = 0.821$), indicating no effect of zolpidem on TMS metrics of $GABA_A$-mediated inhibition (Fig. 6.9B-C).

Lastly, I studied the effect of TMS metrics of $GABA_B$-mediated inhibition: LICI 150ms. As with SICI, I calculated the ratio between the mean amplitude of the paired-pulse and the single-pulse MEP, so that a smaller value represents more inhibition. I then compared these values between treatment conditions using a two-way repeated measures ANOVA with within-subject factors of treatment (zolpidem, placebo) and time (baseline, 1.6h, 2.35h, 3.35h). I found a significant effect of time ($F(1.943, 33.04) = 5.936$, $p = 0.007$), but no significant effect of treatment ($F(1,17) = 0.090$, $p = 0.768$) and no significant time x treatment interaction ($F(2.664, 45.28) = 0.881$, $p = 0.448$), indicating no significant effect of zolpidem on TMS metrics of $GABA_B$-mediated inhibition (Fig. 6.9D).

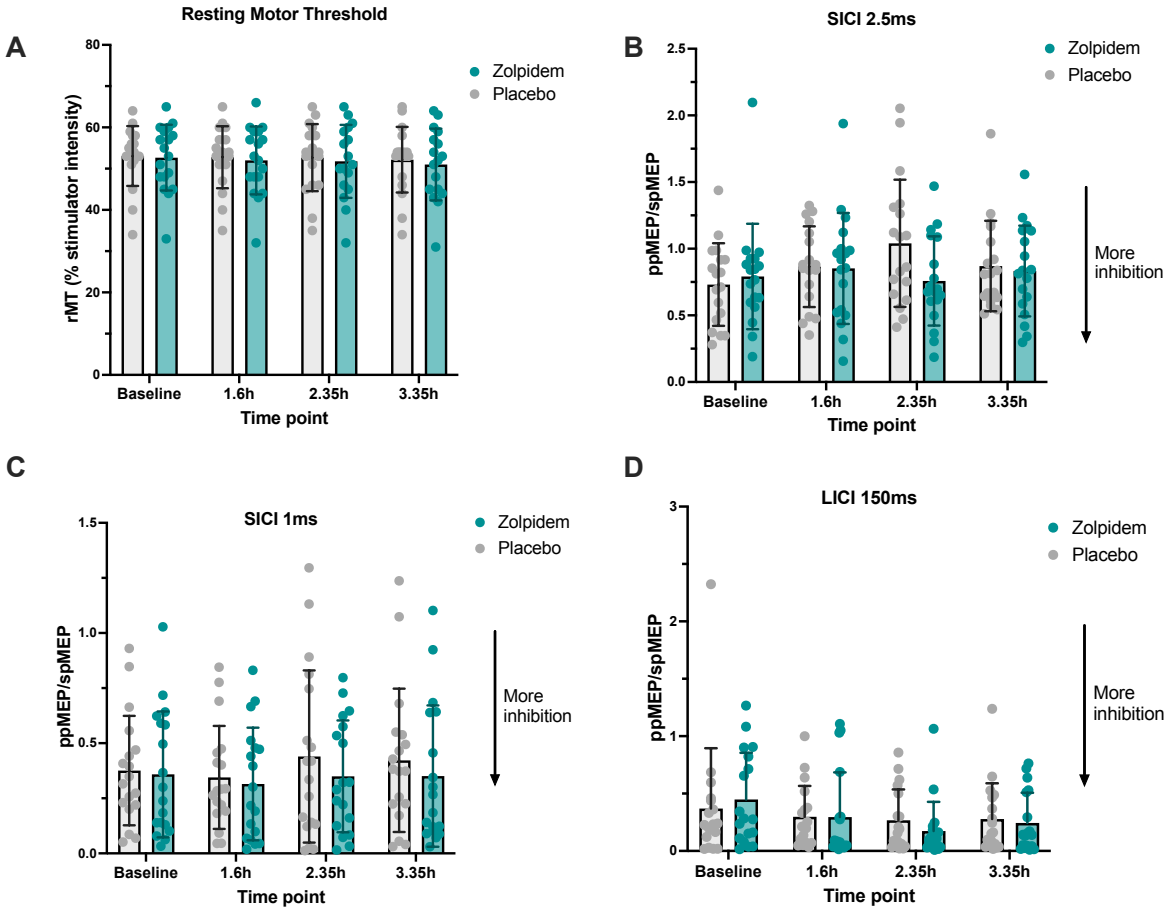


Fig. 6.9. **The effect of zolpidem on TMS metrics.** Zolpidem did not significantly affect measurements of cortical excitability (rMT (A)), $GABA_A$-mediated inhibition (SICI 2.5ms (B); SICI 1ms: (C)) and $GABA_B$-mediated inhibition (LICI 150ms (D))

6.3.6 Zolpidem significantly impaired performance on the AVLT, but not the Digit Span task

I then studied whether zolpidem affected working memory on two tasks: the digit span, investigating very short-term working memory, and the Rey Auditory Verbal Learning Test (AVLT), investigating verbal working memory. To evaluate the performance on the digit span task during each session, I recorded the participants' highest number of correctly recalled digits in each condition, forwards (Fig. 6.10A) and backwards (Fig. 6.10B), and compared these numbers between treatment conditions using paired t-tests. I found no significant difference on digit span performance between treatment conditions (forwards: $t(19) = 0.137$, $p = 0.893$; backwards $t(19) = 0.335$, $p = 0.741$).

To evaluate the performance on the AVLT during each session, I recorded the participants' number of correctly recalled words during the five learning repeats (R1 – R5), as well as the non-repeat trials (immediate – after R5; delayed – 30 minutes after R5). I used a two-way repeated-measures ANOVA with within-factors of treatment (zolpidem, placebo) and time (R1, R2, R3, R4, R5, immediate, delayed) to investigate the effect of zolpidem on AVLT (Fig. 6.10C). I found a significant effect of treatment ($F(1, 19) = 6.976$, $p = 0.016$) and time ($F(2.458, 46.69) = 61.40$, $p < 0.0001$), but no significant treatment x time interaction ($F(3.648, 69.30) = 0.857$, $p = 0.486$). There was no significant effect of session on AVLT performance (two-way repeated-measures ANOVA with within-factors of session (session 1, session 2) and time (R1, R2, R3, R4, R5, immediate, delayed): no significant effect of session ($F(1, 19) = 0.201$, $p = 0.659$), time ($F(2.458, 46.69) = 61.40$, $p < 0.0001$) or time x session interaction ($F(3.755, 71.34) = 0.1.386$, $p = 0.249$).

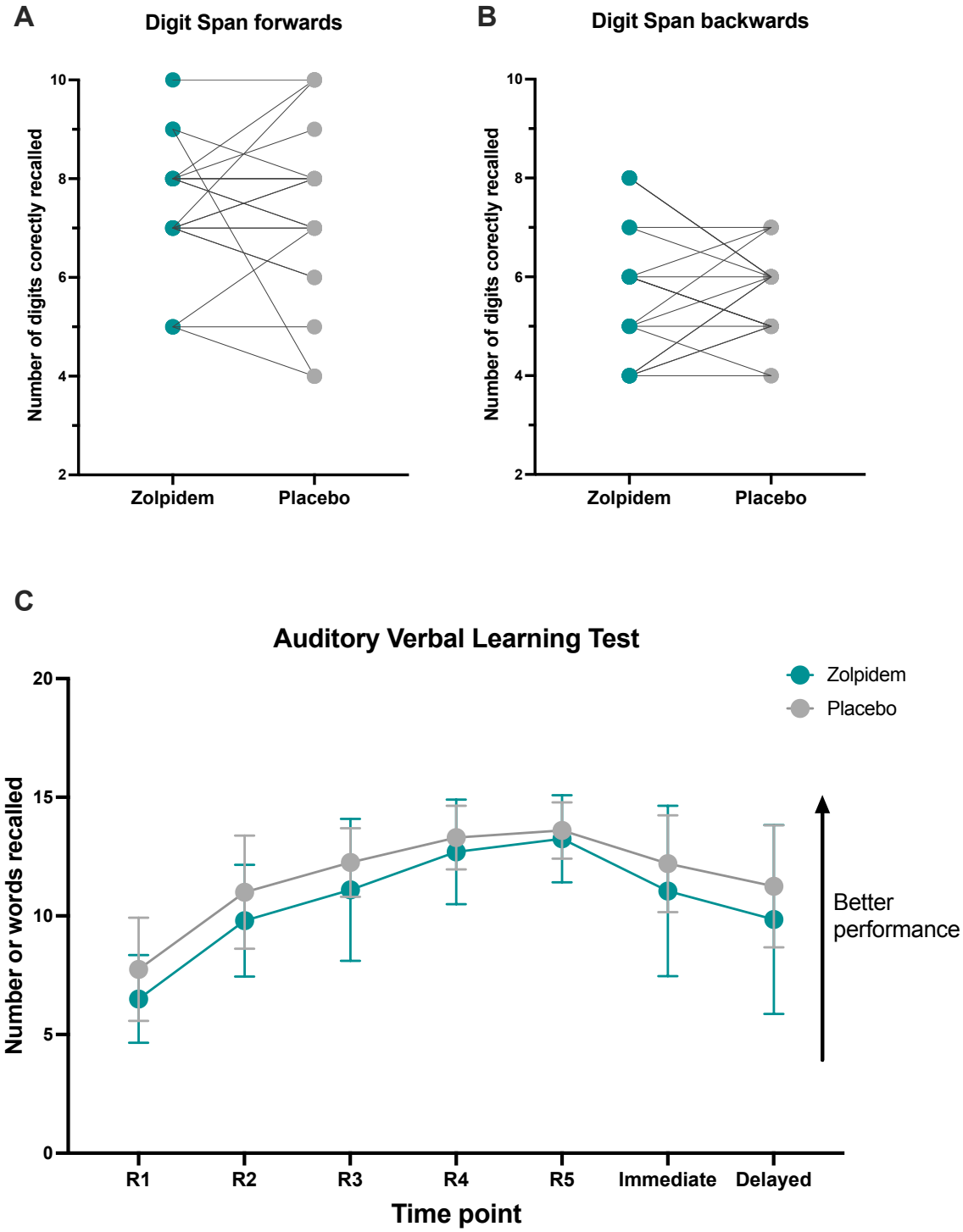


Fig. 6.10. **Performance on working memory tests**: digit span forwards (A) and backwards (B) and AVLT (C). Zolpidem significantly impaired performance on the AVLT but did not significantly affect performance on the digit span

6.3.7 Zolpidem significantly decreased participants' alertness and contentedness

Lastly, I investigated how zolpidem affected the participants' mood. As previously described, the 16 BLVAS scales were loaded onto three mood components: alertness (alert, strong, clear-headed, well-coordinated, energetic, quick-witted, attentive, proficient and interested), contentedness (contented, tranquil, happy, sociable and friendly) and calmness (calm and relaxed). The mean values for each mood component were calculated on each session at 4 time points: before treatment administration (baseline), 1.6h, 2.35h and 3.35h after treatment administration. For each mood component, I investigated the effect of zolpidem using a mixed-effects model (restricted maximum likelihood) with within-subject fixed effects of treatment (zolpidem, placebo) and time (baseline, 1.6h, 2.35h, 3.35h).

On alertness, I found a significant effect of both treatment and time (treatment: $F(1, 19) = 18.52$, $p = 0.0004$; time: $F(2.421, 46.00) = 23.56$, $p < 0.0001$) and a significant treatment x time interaction ($F(2.099, 39.19) = 8.287$, $p = 0.0008$; Fig. 6.11A). On contentedness, I found a significant effect of time ($F(2.619, 49.75) = 9.702$, $p < 0.0001$) and a significant time x treatment interaction ($F(2.367, 44.19) = 5.083$, $p = 0.0073$), but no significant effects of treatment ($F(1, 19) = 4.342$, $p = 0.051$; Fig. 6.11B). On calmness, I found no significant effect of treatment ($F(1,19) = 0.013$, $p = 0.909$), no significant effect of time ($F(2.013,38.25) = 2.207$, $p = 0.124$) and no significant time x treatment interaction ($F(2.371, 44.25) = 0.561$, $p = 0.603$; Fig. 6.11C). This indicates that zolpidem significantly decreased alertness and contentedness compared to placebo but had no significant effect on calmness.

Lastly, I investigated whether these changes in mood with zolpidem might arise from differences in mood before treatment administration using a two-way repeated-measures ANOVA with factors of treatment (zolpidem, placebo) and component (alertness, calmness, contentedness). At baseline, there was no significant effect of treatment ($F(1,19) = 0.012$, $p = 0.914$), no significant effect of component ($F(1.185, 22.52) = 2.019$, $p = 0.168$) and no treatment x component interaction ($F(1.275, 24.23) = 0.896$, $p = 0.378$; Fig. 6.11D).

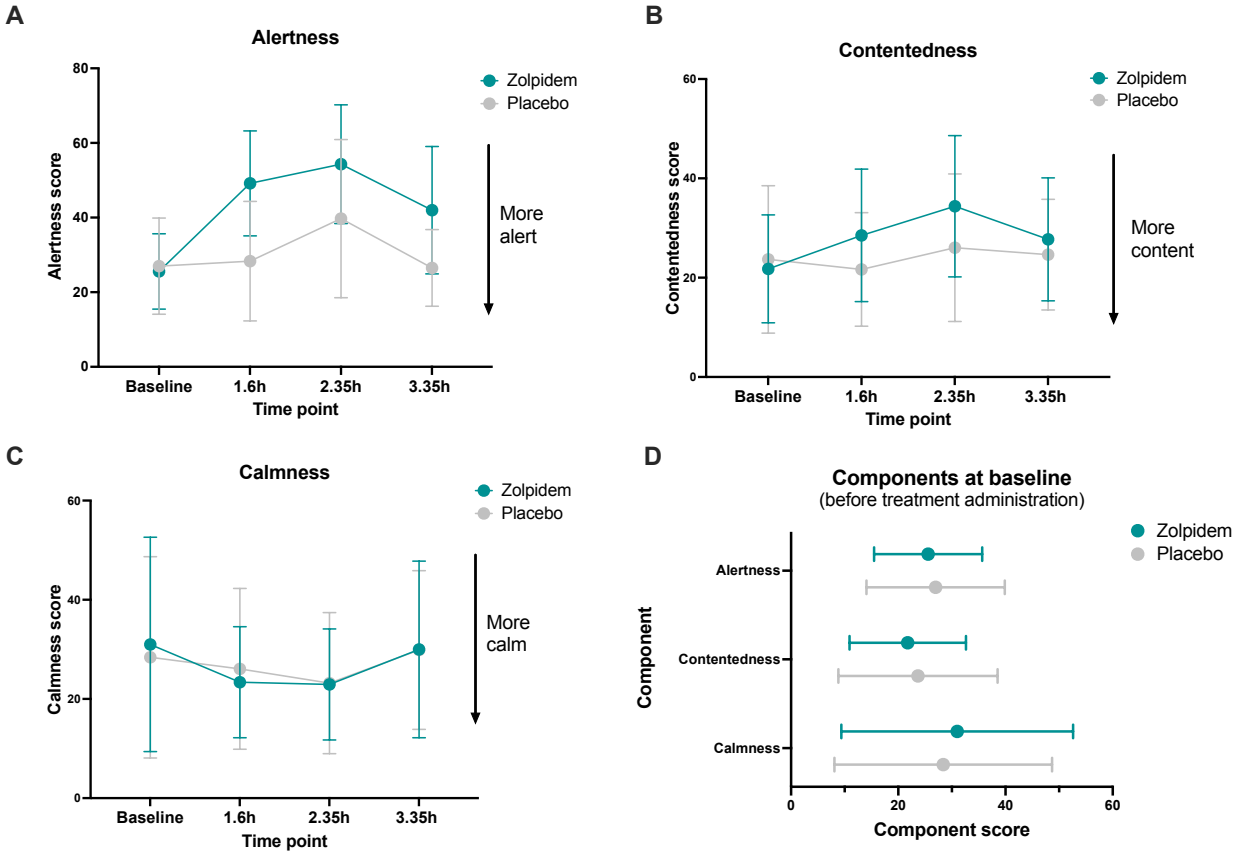


Fig. 6.11. **The effect of zolpidem on mood components**. Zolpidem significantly decreased the participants' alertness (A), but not their contentedness (B) and calmness (C). There were no differences in the mood before treatment administration (D)

6.4 Discussion

Here, I investigated the effect of the $GABA_A$ receptor positive allosteric modulator, *zolpidem*, on motor learning, tactile discrimination and TMS electrophysiological measurements in young, healthy volunteers. I found that increasing GABA-mediated inhibition *via* zolpidem led to significant impairments in response time, motor learning and tactile discrimination. I also found significant impairments in verbal working memory and decreased alertness after zolpidem administration. There were no significant differences in either TMS cortical excitability metrics or GABAergic inhibition metrics between treatment conditions.

6.4.1 The effect of zolpidem on behavioural metrics

6.4.1.1 Zolpidem was associated with slower response times to visual stimuli

First, I investigated whether zolpidem significantly impaired motor execution by measuring participants' performance on the action selection task. I found significantly slower response times during execute blocks and significantly slower action-selection times (select – execute RTs) during the zolpidem session compared to placebo. This finding is consistent with previous reports of zolpidem-related impairments in motor behaviour; zolpidem has been associated with slower response times, impaired visuomotor coordination (grooved pegboard task) and impaired driving skills at 1-2 hours post-administration (Otmani *et al.*, 2008; Bocca *et al.*, 2011; Kleykamp *et al.*, 2012; Pilli *et al.*, 2013; Neylan *et al.*, 2020). $GABA_A$ receptors are highly expressed in the cerebral cortex (Sequeira *et al.*, 2019); by positively modulating their activity, zolpidem could potentially decrease the processing speed of the visual stimuli, connectivity between the visual and motor areas, and/or motor planning and motor execution, ultimately leading to slower

response times in response to visual stimuli. Zolpidem also increases beta-band activity (Patat *et al.*, 1994); beta-band oscillatory activity is an anti-kinetic rhythm (Engel & Fries, 2010) and its zolpidem-induced increase in power might mediate the drug's impairment in motor execution. Zolpidem also decreases participants' alertness, so the slower response times might also be linked to decreased attention during task performance.

6.4.1.2 Zolpidem impaired SRTT task performance

I then investigated the effect of zolpidem on motor sequence learning, by comparing participants' performance on the SRTT between treatment conditions. I found that zolpidem was associated with significantly slower response times during sequence blocks, although this effect did not persist when response times during sequence blocks were normalised to the response times during the first random block. This suggests that, while participants' response times during sequence learning blocks were slower during the zolpidem session, this may have been a result of zolpidem slowing down overall response times rather than impairing motor learning.

When controlling for an order effect, I found a significant treatment x learning x order interaction, indicating the effect of zolpidem on learning was different in the group that had zolpidem during the first session compared to the group that had placebo first. I then further dissected this by comparing participants' learning between treatment conditions for each session. I found that there was no significant learning in the zolpidem session, regardless of whether zolpidem was administered on the first or the second session. I found significant learning in both placebo sessions. When treatment conditions are compared for each session, there is only a significant effect of treatment in the second session. One possible explanation is that perhaps participants were more excited to

complete the task in the first session when it was novel, so their response times are faster even if they do not significantly learn the sequence; then they may have been less motivated in the second session since they had already been familiarised with the task, so their response times are much slower. Another possible explanation is that the second sequence was more difficult to learn, although they both contained the same number of buttons in total and the same number of buttons for each digit (session 1: 342131241324; session 2: 231434124213).

One previous study found that zolpidem impaired sleep-dependent motor skill consolidation, likely through its impact on the duration of different sleep stages and sleep composition (Morgan *et al.*, 2010), but no studies have looked at the effect of zolpidem on motor sequence learning shortly after administration. I are therefore the first to report the effect of zolpidem on SRTT task performance: response times were significantly slower during the sequence blocks, but this effect may be due to overall impairments in response times rather than impaired motor learning. It is possible that, while $GABA_A$ receptors containing α1 subunits are implicated in the processes related to motor execution, they might not be involved in the mechanisms related to motor learning and plasticity. Indeed, administration of zolpidem did not significantly affect TMS-induced LTP-like plasticity in humans (Lücke *et al.*, 2014).

6.4.1.3 Zolpidem significantly impaired retention of visuomotor adaptation skills

I then investigated the effect of zolpidem on visuomotor adaptation to perturbations in sensorimotor mapping, by comparing the participants' performance on the VMA task between treatment conditions. I found no significant effect of zolpidem on the behavioural metrics from the VMA main task, indicating that zolpidem did not change the acquisition of visuomotor adaptation skills. However, I found a significant effect of zolpidem on the VMA washout task, indicating that zolpidem significantly impaired the retention of visuomotor adaptation skills. One previous study showed that zolpidem was associated with impaired visuomotor coordination measured using the grooved pegboard task (Otmani *et al.*, 2008), however ours is the first study to report the effect of zolpidem on visuomotor adaptation. There are distinct circuits involved in visuomotor adaptation and retention; acquisition of visuomotor adaptation skills relies on a network including both cortical and subcortical motor areas, of which the cerebellum plays an important role (Seidler & Noll, 2008; Jenkinson & Miall, 2010; Hardwick *et al.*, 2013); meanwhile, retention of visuomotor adaptation skills is thought to mostly rely on M1 (Richardson *et al.*, 2006; Hadipour-Niktarash *et al.*, 2007; Galea *et al.*, 2011; Nettekoven *et al.*, 2021). One possible explanation is that zolpidem affected the activity of M1 and not the cerebellum, although the α1 subunit-containing $GABA_A$ receptors are highly expressed in both the cortical and cerebellar cortices (Sequeira *et al.*, 2019). Alternatively, since adaptation relies on a network of multiple brain areas, there might be multiple compensatory mechanisms available when the activity of the network is disrupted; however, since retention relies mostly on M1, there might be fewer options to compensate for M1 disruptions.

6.4.1.4 Zolpidem significantly impaired tactile discrimination

I then investigated the effect of zolpidem on tactile discrimination, by comparing the participants' performance on the TOJ task between treatment conditions. Since higher GABAergic inhibition is associated with better performance on tactile discrimination tasks (Puts *et al.*, 2011; Kolasinski *et al.*, 2017) and higher baseline sensorimotor [GABA] is associated with better learning in a tactile perception task (Heba *et al.*, 2016), I hypothesised that increasing GABAergic inhibition *via* zolpidem would lead to improved tactile discrimination. Contrary to our hypothesis, I found that zolpidem significantly impaired tactile discrimination, as it was associated with higher values for the just-noticeable difference (JND). However, this might be because zolpidem also significantly impaired the overall TOJ task performance: the data's goodness-of-fit to the logistic functional was significantly lower with zolpidem compared to placebo. Additionally, two participants were removed from the analysis due to low R^2 values ($R^2 < 0.4$); one participant only had a low R^2 on the zolpidem session, while the other had low R^2s on both sessions. One more participant was removed from the analysis as their R^2 was revealed to be an outlier using the Grubbs test; this value was also for the zolpidem session. It is possible that zolpidem's effect on alertness impaired the participants' ability to correctly perform the task, shown by the significantly decreased R^2 values. The JND values are then calculated based on the logistic function fit, so the decreased goodness-of-fit is likely to have affected the calculation of JND and could potentially hide a GABAergic mediated improvement in tactile discrimination. Only one previous study investigated the effect of pharmacologically increasing $GABA_A$ mediated inhibition on tactile perception. They showed impairments in tactile task performance, with longer response times and higher variability in response times after administration of $GABA_A$ receptor positive allosteric

modulator alprazolam, but they did not investigate tactile discrimination (Fioravanti *et al.*, 2019). Future studies investigating the role of GABAergic inhibition in touch perception should consider using smaller doses of $GABA_A$ receptor modulators to minimise effects on alertness and enable the acquisition of good quality data from touch perception tasks.

6.4.1.5 Zolpidem significantly impaired verbal working memory and recall

Learning new motor skills involves working memory processes (Seidler *et al.*, 2012) and previous studies show impairments in working memory and memory recall after administration of 10 – 15mg of zolpidem (Mintzer & Griffiths, 1999; Stoops & Rush, 2003; Otmani *et al.*, 2008; Kleykamp *et al.*, 2012). Therefore, I investigated the effect of zolpidem on working memory to determine whether our smaller dose (5mg) still impaired working memory and to be able to control for this potential effect on motor learning. I found no significant difference in the maximum length of digits correctly recalled in the digit span task (forwards or backwards) between zolpidem and placebo sessions. However, I found that zolpidem significantly decreased the number of total words recalled during the AVLT, both during list repeats (participants listened to the list and were then asked to repeat as many words as they could remember) and immediate and delayed recalls (participants were asked to repeat as many words as they could remember from the list, without hearing the list again). These results are consistent with previous reports of zolpidem 10mg significantly impairing performance on a word recall test, but not on the digit span test, at 1h and 2h post-administration (Troy *et al.*, 2000; Neylan *et al.*, 2020); 20mg of zolpidem significantly impaired performance on both working memory tests, indicating that the behavioural effects are dose-dependent (Troy *et al.*, 2000).

Zolpidem is thought to affect the encoding step rather than the recall of the information, as the recall of information encoded prior to drug administration is not significantly affected by zolpidem (Mintzer & Griffiths, 1999; Neylan *et al.*, 2020).

The mechanisms through which zolpidem exerts its effect on working memory are incompletely understood, as higher MRS-derived GABA in the dorsolateral prefrontal cortex (DLPFC) has been linked to better working memory in healthy people (Yoon *et al.*, 2016; Ragland *et al.*, 2020). This relationship between higher inhibition and improvements in behaviour is hypothesised to be mediated through similar mechanisms as those in sensory cortices, where higher inhibition is linked to better sensory discrimination. However, I found the opposite in this study: increasing GABAergic inhibition was associated with impairments in working memory. There might be several reasons for this. The relationship found by (Yoon *et al.*, 2016) was region-specific: only [GABA] in the DLPFC and not other brain areas was associated with better working memory. Since zolpidem is a whole-brain intervention, its effect on brain areas other than the DLPFC may have contributed to the behavioural impairments. Moreover, MRS-derived [GABA] measures the total concentration of GABA in a brain area, which may reflect a measure of extrasynaptic GABAergic inhibition (Stagg *et al.*, 2011*b*); zolpidem mostly binds to synaptic GABAergic receptors, the activity of which is not reflected in MRS-derived metrics (Stagg *et al.*, 2011*b*; Tremblay *et al.*, 2013; Mooney *et al.*, 2017). It is also possible that the deficits in working memory arise from a zolpidem-induced decrease in alertness and attention.

It is unclear why zolpidem affects performance on the AVLT and not the digit span task. Previous studies show significant correlations between participants' performance on the

digit span and the AVLT (Khosravi Fard *et al.*, 2016), but the digit span is a more simple task probing very short-term working memory and memory capacity (Richardson, 2007; Woods *et al.*, 2011). Meanwhile, AVLT assesses more complex working memory processes including encoding, storing and retrieving information at longer delays than the digit span (minutes rather than seconds) and may provide a more comprehensive view of working memory and learning (Geffen *et al.*, 1990). Since the digit span is less cognitively demanding, participants may be able to adequately complete it despite increased GABAergic inhibition or, if compensatory mechanisms for the increased GABAergic inhibition are employed, they may be more effective. Additionally, it is possible that the digit span precision of 1 digit is not enough to reveal small drug effects on working memory, as it is similar to the standard deviation of the metric (Ardila, 2007; Woods *et al.*, 2011); the maximum span recorded also does not provide any information on the performance throughout the task.

6.4.2 The effect of zolpidem on TMS metrics

6.4.2.1 Zolpidem did not significantly affect rMT, a TMS metric of cortical excitability

I then used TMS to investigate how zolpidem changed cortical excitability and electrophysiological measurements of GABAergic inhibition. I first compared the resting motor threshold (rMT), the stimulation intensity required to elicit a 1mV MEP (see Methods), between treatment conditions. I found no significant difference in rMT, indicating that zolpidem did not significantly affect cortical excitability. This finding is consistent with previous studies that also did not find a significant change in 50 µV rMT, the 1mV rMT or the aMT after administration of 10mg of zolpidem (Di Lazzaro *et al.*, 2006;

Teo *et al.*, 2009). However, these studies did not have a placebo arm and compared the post-administration time points with the baseline (pre-administration) measurement. Of these previous studies, one found a significant increase in the 1mV rMT with time, but not a time x treatment interaction; it is possible that this increase in the 1mV rMT was related to decreases in cortical excitability since all the studied drugs (zolpidem, diazepam, lorazepam) were $GABA_A$ receptor positive allosteric modulators (Di Lazzaro *et al.*, 2006). I did not find any time effects on 1mV rMT, but it is possible that our smaller dose of zolpidem (5mg compared to 10mg in previous studies) did not lead to any significant changes.

6.4.2.2 Zolpidem did not significantly change SICI and LICI, TMS metrics of GABAergic inhibition

I then compared three different TMS metrics of GABAergic inhibition (SICI 2.5ms, SICI 1ms and LICI 150ms) between treatment conditions and found that zolpidem did not significantly affect any of them. I used these three metrics because they have been linked to different types of GABA-mediated inhibition; specifically, SICI 2.5ms is thought to reflect synaptic $GABA_A$ receptor activity, SICI 1ms extrasynaptic $GABA_A$ receptor activity and LICI 150ms $GABA_B$ receptor activity (Ziemann *et al.*, 1996*b*; Ilić *et al.*, 2002; Roshan *et al.*, 2003; Ziemann, 2004; McDonnell *et al.*, 2006; Ni *et al.*, 2007; Stagg *et al.*, 2011*b*). Our results here are consistent with previous findings: neither SICI 3ms, nor LICI 100ms changed significantly 2 hours after administration of 10mg of zolpidem (Di Lazzaro *et al.*, 2006, 2007; Teo *et al.*, 2009). Since zolpidem is a specific positive modulator for $GABA_A$ receptors containing the α1 subunit, it has been hypothesised that SICI is mediated by non-α1 $GABA_A$ receptors, like receptors containing subunits α2 or α3. Notably, these

studies did not have a placebo arm and TMS data was only collected at 1 timepoint when zolpidem would reasonably be expected to still be at an effective plasma concentration (2 hours post drug administration), with all changes in TMS metrics calculated compared to the pre-administration time point. In two of the studies, the measurements were calculated from only 5 measurements (either 5 single test pulses or 5 paired pulses) per condition, which is very little considering that TMS is a noisy technique (Di Lazzaro *et al.*, 2006, 2007; Beaulieu *et al.*, 2017). Only one early study found significant increases in SICI 3ms and LICI 150ms at 1h after administration of zolpidem, but the intensity of the test pulse was not adjusted to match the amplitude of the test MEP between measurements, which may have biased the paired-pulse TMS metrics; the baseline measurements were also acquired on different days compared to the sessions on which the drugs were administered (Mohammadi *et al.*, 2006).

6.4.3 Zolpidem affected the participants' mood, significantly decreasing alertness and contentedness

Lastly, I investigated the effect of zolpidem on mood by comparing participants' responses to a BLVAS between treatment conditions. I found a significant decrease in alertness and contentedness in the zolpidem session compared to placebo, but no significant effect of zolpidem on calmness. This is not surprising considering the clinical use of zolpidem as a sleeping pill, but I tried to minimise these effects by administering a 5mg dose that was lower than both the clinically recommended dose and the dose used in previous studies (10mg). It is well known that zolpidem increases sleepiness and fatigue, and decreases attention in a dose-dependent manner (Troy *et al.*, 2000; Stoops & Rush, 2003; Teo *et al.*, 2009; de Haas *et al.*, 2010). Its effect on contentedness is more surprising, as zolpidem is

known to have a minor anxiolytic effect, likely mediated by its weak affinity to $GABA_A$ receptors containing subunits α2 or α3 when administered in higher concentrations (Langtry & Benfield, 1990; Bailey *et al.*, 2009; Fitzgerald *et al.*, 2014). One possible explanation would be that participants became frustrated at their impaired performance in most sensorimotor tasks, which made them less content. However, zolpidem has been shown to disrupt metacognition: participants are not aware of the magnitude of behavioural impairments and tend to overestimate their performance on sensorimotor tasks (Stoops & Rush, 2003; Kleykamp *et al.*, 2012).

6.5 Conclusion

I conducted a within-subject, double-blind, placebo-controlled study to probe the contribution of $GABA_A$ receptors in motor learning and brain chemistry. I used zolpidem, a specific α1 $GABA_A$ receptor positive allosteric modulator, as a pharmacological intervention, and compared participants' performance on sensorimotor tasks and working memory tests, as well as TMS metrics, between zolpidem and placebo. I found that zolpidem significantly impaired performance on the action selection task, the serial reaction time, and the temporal order judgment task. Zolpidem also impaired retention of visuomotor adaptation skills, and verbal working memory, while decreasing alertness. I found no significant changes in TMS metrics with zolpidem. These results are clinically relevant: zolpidem is used as a hypnotic in clinical populations such as stroke survivors, and it could impair their sensorimotor performance and motor skill retention.

CHAPTER 7 - GENERAL DISCUSSION

The aim of this thesis was to investigate the role of inhibition in human motor learning. To this end, I conducted two double-blind, randomized, placebo-controlled experiments to evaluate the role of different types of GABA receptors in motor learning and brain chemistry in young, healthy volunteers. The first experiment was a pharmaco-MRI study investigating the effect of baclofen, a specific $GABA_B$ receptor agonist, on motor learning and MRSI measurements of neurochemicals in motor areas (Chapter 3), task fMRI during motor sequence learning (Chapter 4) and resting-state fMRI (Chapter 5). The second experiment was a pharmaco-TMS study investigating the effect of zolpidem, a specific α1-containing $GABA_A$ receptor positive allosteric modulator, on motor learning and TMS measurements of corticospinal excitability and GABA-mediated inhibition (Chapter 6).

7.1 SUMMARY

In the first chapter of this thesis, I reviewed the role of the primary motor cortex in encoding different movement characteristics, including force, direction, speed and fine motor control. I then discussed the role of oscillatory brain activity in motor control and highlighted the results of non-invasive brain stimulation studies that use transcranial alternating current stimulation (tACS) to entrain the neuronal population in the motor cortex at specific frequencies. Lastly, I discussed the importance of motor learning in human life and reviewed what is known about the role of inhibition in the primary motor cortex in human motor learning. While increasing evidence links lower inhibition with better motor learning, it is still unclear whether this relationship is causal or not. I aimed

to test this in my thesis by using specific GABA drugs to modulate GABA-mediated inhibition and determine the effect of increased GABAergic inhibition on motor learning.

In Chapter 2, I reviewed the methods and techniques used in this thesis to investigate motor learning, brain activity during task performance and brain chemistry. I discussed the usage and underlying network activity for three motor tasks: the action selection task, used to evaluate motor execution and action selection; the serial reaction time task, used to evaluate motor sequence learning; and the visuomotor adaptation task, used to evaluate adaptation to perturbations in visuomotor mapping and the retention of this skill. I described the mechanism of action and usage of functional MRI: task fMRI to determine the patterns of brain activity during task performance and resting-state fMRI to determine functional connectivity between brain areas. I also discussed Magnetic Resonance Spectroscopic Imaging (MRSI), which was used to quantify the GABA concentration in the primary motor and premotor cortices. Lastly, I reviewed how single-pulse and paired-pulse TMS protocols can be used to measure corticospinal excitability and inhibition mediated by different GABA receptor types, as well as the limitations of this technique.

In Chapter 3, I investigated the effect of baclofen on motor learning and GABA concentration in the primary motor cortex and premotor cortex. I found that baclofen significantly impaired performance on a motor sequence learning task, but had no significant effect on response times to visual stimuli or action selection. There was no significant effect of baclofen on visuomotor adaptation. While there was no significant effect of baclofen on pre-learning [GABA], baclofen significantly affected the learning-related dynamics of [GABA] in the left M1 and left premotor cortex.

In Chapter 4, I investigated how increasing GABAergic signalling with baclofen impacted network activity during motor sequence learning. The patterns of brain activity during SRTT task performance were significantly different between treatment conditions. Specifically, I found higher activity in the frontal pole during sequence learning and lower activity in the thalamus and basal ganglia in the baclofen session compared to placebo. In this case, the frontal pole is likely additionally recruited as a compensatory mechanism during learning.

In Chapter 5, I investigated how increasing GABAergic signalling with baclofen impacted network activity at rest. I found decreased functional connectivity in resting-state networks (sensorimotor, visual and default mode networks) in the baclofen session compared to placebo. Voxel-wise analysis revealed decreased within-network connectivity in specific areas of both the visual and sensorimotor networks with baclofen. Sensorimotor [GABA] was not changed by baclofen administration and did not significantly correlate with the strength of functional connectivity in the motor network.

In Chapter 6, I investigated the effect of zolpidem on motor learning and TMS metrics of GABA-mediated inhibition. I found that zolpidem significantly impaired reaction times to visual stimuli and action selection times. Zolpidem also significantly impacted both performance on the serial reaction time task and tactile discrimination as measured by the temporal order judgment task. Zolpidem significantly impaired retention of visuomotor adaptation skills. I found no significant changes in TMS metrics with zolpidem. Lastly, zolpidem significantly impaired verbal working memory and participants' alertness.

Taken together, these experiments strengthen our understanding of the contribution of different GABA-receptors to human motor learning and plasticity. $GABA_B$-receptors are involved in the process of motor learning, as I saw behavioural impairments in performance on a motor sequence learning task after baclofen administration, with no significant effects on pure reaction times, working memory or alertness. The role of $GABA_A$-receptors is more difficult to interpret, as zolpidem significantly decreased alertness, working memory and reaction times, all of which contributed to the impaired performance on the serial reaction time task. However, despite this confound, these results might have clinical relevance, since both baclofen and zolpidem are commonly used in clinical practice as a muscle relaxant and sleeping pill, respectively. Whether their administration also impairs the motor rehabilitation of stroke survivors remains to be studied.

7.2 Main Conclusions

1. Increasing $GABA_B$-mediated inhibition with baclofen led to impairments in performance on a motor sequence learning task, by slowing down response time during sequence blocks, and a significant change in the learning-related [GABA] dynamics in the left primary motor and premotor cortices.

2. With baclofen, there was higher activity in the frontal pole during motor sequence learning and lower activity in the thalamus and basal ganglia compared to placebo. The higher activity in the frontal pole is likely due to the recruitment of additional brain areas as a compensatory mechanism.

3. Functional connectivity in the three resting-state networks of interest (sensorimotor, visual and default mode network) was significantly decreased in the baclofen session compared to placebo. This was associated with areas of decreased within-network functional connectivity in the visual and sensorimotor network, as revealed by voxel-wise analysis.

4. Increasing $GABA_A$-mediated inhibition led to significant impairments in behaviour and mood. It significantly slowed down reaction times and impaired retention of visuomotor skills, working memory and alertness. No significant effects of zolpidem on TMS metrics were found.

7.3 General limitations of pharmacological studies

The limitations of the methods employed in the two pharmacological studies have been discussed in more detail in their respective chapters. Here, I will briefly address the limitations of pharmacological studies.

Although the pharmacological interventions I used here are highly receptor-specific, they are not region-specific, due to the widespread expression of both $GABA_A$ and $GABA_B$ receptors across the brain. Therefore, it is impossible to know whether the effect of the drug on non-motor areas might have influenced the results reported here. I was able to measure some non-motor behaviour, such as alertness and working memory, which could have confounded performance on the motor sequence task. However, since the drugs are whole-brain interventions, it is impossible to eliminate these effects altogether. This was particularly important for the zolpidem study, in which the 5mg of zolpidem significantly impaired response times, working memory and alertness. It is possible that a smaller dose

could have facilitated our investigation of motor learning without the presence of these effects.

Additionally, the pharmacodynamics and pharmacokinetics of the drug can present some between-subject variability, which could influence the behavioural effects. The drugs were administered at the same dose to all participants, but I did not control for changes in body weight between participants. Since both our sample sizes were relatively small (15 people for the baclofen project and 20 for the zolpidem project), it is not impossible that this sample is not representative of the whole population in terms of drug effects and metabolism.

Within-subject studies have the advantage of comparing the same participant's performance between a drug and placebo conditions, but other sources of variation might still exist between sessions: duration and quality of sleep, familiarization with the study protocol on the second session, physiological noise or other personal issues that could have occurred between the two sessions. I asked participants to sleep well the night before each session and not have more than 1 cup of caffeinated beverage before the session, but it is impossible to confirm they did as instructed.

The pharmacological agents used here are commonly administered to patients. Since they impair motor behaviour in healthy people, it is important to investigate whether they might also impair the motor rehabilitation of clinical populations. There are differences between the drug administration here and the one in patients which could influence the results. For example, in clinical practice baclofen is usually given in multiple doses (4-5) throughout the day, which might increase patients' tolerability to it compared to the single dose in our study. Zolpidem is normally administered before bed, so the patients

would not complete any rehabilitation procedures while on zolpidem; the effects of zolpidem after waking are reported to be reasonably small. Lastly, the effect of neurotransmitters in the brain can be different under pathological conditions compared to health, so the drugs might show different effects.

7.4 FUTURE DIRECTIONS

The results of this thesis helped further our understanding of the role of inhibition and the contribution of different GABA receptors to human motor learning. Several avenues of research remain to be pursued.

7.4.1 The genetic basis for inhibition in the brain

The activity of brain networks is crucial for correct brain function and behaviour, and the strength of within-network functional connectivity depends on [GABA] (Kapogiannis *et al.*, 2013; Stagg *et al.*, 2014), with higher functional connectivity being associated with lower [GABA] in key network regions. However, the genetic factors underlying the physiological variations in [GABA] and how they might influence functional connectivity in healthy people are incompletely understood. I have an approved application to use UK Biobank data to investigate the role of genetic polymorphisms in GABAergic genes on the functional connectivity of resting-state networks. For each participant, I will use genetic data to identify the participant's alleles for two SNPs in GABA-related genes (*GAD1*, *GABRA5*), and neuroimaging data to identify their sensorimotor and default mode networks. I hypothesise that the genetic polymorphisms associated with lower GABAergic neurotransmission will be associated with higher within-network functional connectivity.

I am currently writing a registered report outlining the plans of this analysis to soon submit for peer-review in a scientific journal.

7.4.2 The effect of baclofen on oscillatory activity

My previous work includes studying the effects of baclofen using TMS (Johnstone *et al.*, 2021*b*) and, in this thesis, I explored the effects of baclofen using neuroimaging techniques. The effects of baclofen on oscillatory brain activity and how that relates to behavioural metrics are still unknown. This could be further investigated by conducting a double-blind, randomised, placebo-controlled study using behavioural tasks and magnetoencephalography (MEG). Using MEG, I would be able to identify baclofen-related changes in the power of beta and gamma oscillations, as well as changes in event-related synchronisations and desynchronisations in these frequency bands.

7.4.3 The effect of baclofen on the motor recovery of stroke survivors

As mentioned previously, it is crucial to determine whether the administration of GABA agonists impairs the motor rehabilitation of stroke survivors. Answering this question could be achieved by conducting an epidemiological study investigating differences in clinical outcomes between patients prescribed GABA-modulating drugs, such as baclofen, and those who were not, using available large databases (e.g. the South London Stroke Register; OxVasc). The results of this study would allow clinicians to reassess the risk-benefit analysis of administering the drug to patients completing motor rehabilitation.

7.4.4 The role of inhibition throughout stroke recovery – an animal neuroimaging study

Of the neural mechanisms of post-stroke recovery, excitation–inhibition balance is critical and GABA is a key player (Johnstone *et al.*, 2018). In my published review, I discussed the complex changes in GABAergic regulation characterising each stroke stage in humans and rodents, and their relationship to motor recovery (Grigoras & Stagg, 2021). Invasive electrophysiology methods in animal models revealed distinct pathogenic mechanisms for each stroke stage (Fig. 7.1), but validating this timeline has been hindered by the paucity of cross-species tools (Fig. 7.1-Q1).

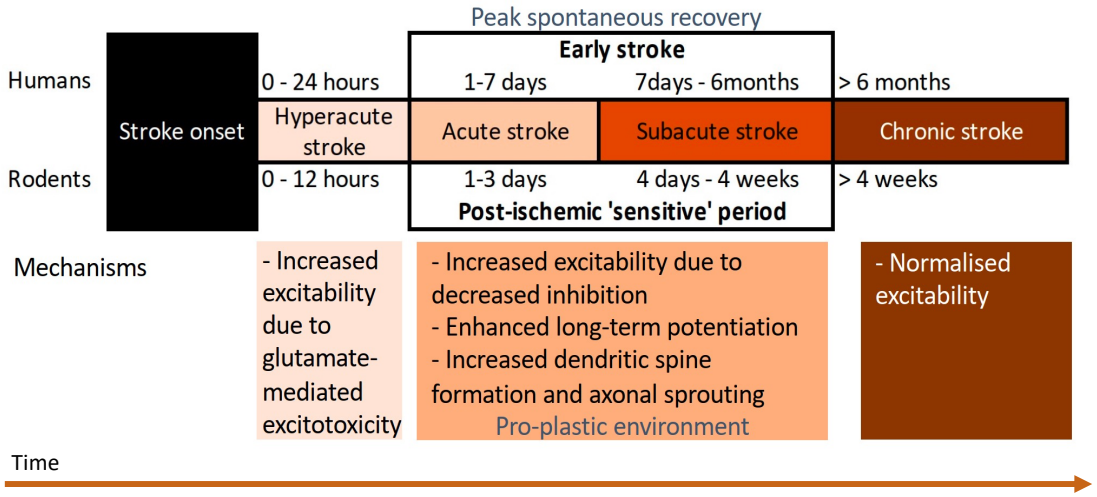
I will investigate the stage-specific changes in excitation-inhibition balance using a novel non-invasive cross-species technique [Magnetic Resonance Spectroscopy (MRS)], similar to the MRSI data I acquired in humans and presented in Chapter 3 (Stagg & Rothman, 2014; Kolasinski *et al.*, 2019*a*) (Fig. 7.2).

This experiment will be the first longitudinal *in vivo* MRS project in rodents, characterising the neurochemical balance at each stroke stage. My department reviewed this project and awarded me £13,000 for research costs (WIN Seed Grant 2021).

I will learn to perform the endothelin-1 stroke model, which is reliable and reproducible (Soleman *et al.*, 2010), with good long-term survival. MRS scans will be acquired with the help of the preclinical neuroimaging team at the WIN.

My first hypothesis is that GABA levels will decrease in early stroke and later normalize in chronic stroke. My second hypothesis is that lower GABA concentrations will be associated with better motor recovery.

Understanding the neurochemical milieu of stroke recovery will help the development of new stage-specific therapy options: either the optimisation of existing brain stimulation procedures or the discovery of novel pharmacological interventions. The common imaging pipeline will help translation by allowing us to compare rodent and human MRS data.



Q1. Can this stroke recovery timeline be validated in a longitudinal study using a translatable technique?

Fig. 7.1. **Putative changes in excitation-inhibition balance during stroke recovery**

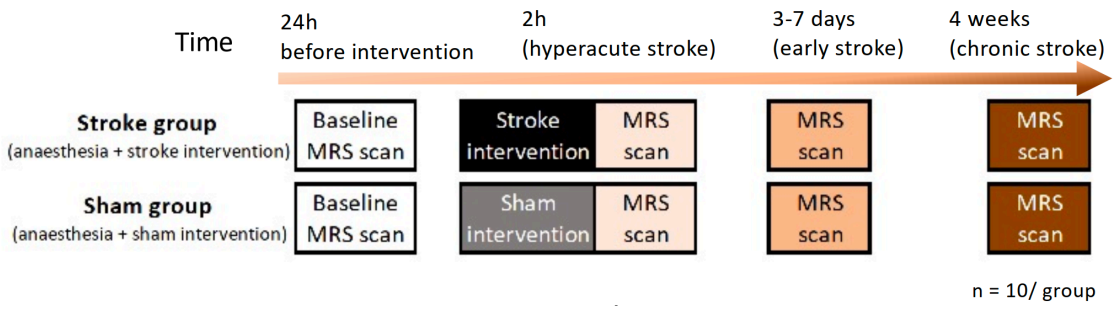


Fig. 7.2. **Experimental design**

7.5 Conclusion

In this thesis, I set out to investigate the role of GABA-mediated inhibition in human motor learning by conducting two within-subject, randomized, double-blind pharmacological studies using specific GABA receptor modulators. I found that baclofen, a $GABA_B$ receptor agonist, significantly impaired performance on a motor sequence learning task and changed the learning-related [GABA] dynamics in the left M1 and premotor cortex. Additionally, baclofen was associated with the recruitment of frontal areas during motor sequence learning and decreased functional connectivity in resting-state networks. Zolpidem, a $GABA_A$ receptor positive allosteric modulator, significantly slowed down response times and impaired the retention of visuomotor adaptation skills. No effects of zolpidem were detected on TMS metrics. These results are important not only because they enhance our understanding of the neurophysiological mechanisms underlying human motor learning, but also because they might influence therapeutical decisions. GABA receptor agonists and positive allosteric modulators are commonly administered in clinical practice, and they might be detrimental to the motor rehabilitation of clinical populations.

LIST OF FIGURES

Fig. 2.1. ***Placement of MRSI slab.*** *The MRSI slab was placed over the sensorimotor cortex, as to include the left and right-hand knobs (red arrows)* 50
Fig. 2.2. ***Example of ^{1}H-MRS spectra from one MRSI voxel.*** *(A) GABA/Cre map from one participant. The following LCModel fit is from the voxel highlighted in the black frame. (B) LCModel fitting output for data from voxel in (A). (C) LCModel fit for GABA* 51
Fig. 2.3. ***Schematic of TMS mechanism of action and detection of MEPs using electromyography (EMG)****. Figure created in bioRender* 53
Fig. 3.1 ***Timeline of the testing session****. The pre-scan period consisted of initial paperwork and sensorimotor task training: MRI-safety screening, Edinburgh handedness test, mood questionnaires and task training (action selection [AS], serial reaction time task [SRTT], and visuomotor adaptation [VMA]). During the MRI scan period, participants were first asked again to fill out the MQs at 45 min post-drug administration (PA). Participants then entered the MRI scanner for ~1 hr, during which we recorded: T1 structural scan, resting-state (RS) functional MRI (fMRI), pre-motor-task magnetic resonance spectroscopy imaging (MRSI), fMRI with simultaneous AS task, fMRI with simultaneous SRTT, and post-motor-task MRSI. During the post-scan period, participants filled in the third set of MQs (2h PA), VMA task, CANTAB memory tests, and a shortened version of the SRTT. Adapted from MSc student thesis (Geist 2019)* 63
Fig. 3.2. ***AS instructions and block order****. a) Sample EB instructions; b) Sample SB instructions; c) Block order* 65
Fig. 3.3. ***Serial reaction time task (SRTT)****. a) Rest screen; b) Sample cue; c) Block order* 67
Fig. 3.4. ***Visuomotor adaptation task (VMA).*** *a) Target circle: 8 potential targets (yellow) and cursor (red); b) Cursor trajectory in FB-ON blocks* 69
Fig. 3.5. ***MRSI slabs.*** *(A) Example of GABA map in one participant – voxels that pass quality checks (SNR > 40, CRLB < 50%) in red and excluded voxels in blue. (B) All acquired MRSI slabs cover the left- and right-hand knobs (white arrows)* 72
*Fig. 3.6****. Behaviour on Action Selection Task.*** *No significant differences between baclofen and placebo sessions for response times on execute blocks (A) or action selection times (B)* 74
Fig. 3.7. ***Behaviour on the Serial Reaction Time Task.*** *Motor sequence learning was significantly impaired after baclofen administration compared to placebo* 76
Fig. 3.8. ***Behaviour on the visuomotor adaptation task.*** *No significant difference in mean angular errors during feedback-on blocks (white background) and feedback-off blocks (grey background) between baclofen and placebo session* 78
Fig. 3.9. ***Examples of GABA/tCr concentrations*** *in voxels in the left primary motor cortex (A) and left premotor cortex (B). The anatomical mask for each brain region is shown in blue* 79
Fig. 3.10. ***Pre-task [GABA]*** *did not significantly change in the left primary motor cortex (A) and left premotor cortex (B) after baclofen administration* 79
Fig. 3.11. ***[GABA] before and after motor learning task****. Baclofen significantly affected the pattern of [GABA] change during motor learning* 81
*Fig. 3.12****. Relationship between pre-task [GABA] and response times during random button presses****. Significant correlation between pre-task [GABA] in the left premotor cortex (A) and response times during the placebo session. No significant correlation between pre-task [GABA] in the left M1 and response times (B)* 82
*Fig. 3.13****. Relationship between*** ***Δ[GABA] and a learning metric*** *calculated based on performance during the SRTT* 84
Fig. 4.1. ***Mean activation maps for action-selection (A) and SRTT (B) task performance****. These maps were then used as pre-threshold masks in task group-level analysis* 107
Fig. 4.2. ***Voxels with significantly higher activity during SRTT blocks after baclofen administration compared to placebo.*** *(A) Higher activity in the left frontal pole across all SRTT blocks (random and sequence) with baclofen (i.e. baclofen – placebo); (B) Higher activity in frontal poles (left frontal pole – top; right frontal pole – bottom), paracingulate gyrus and frontal medial cortex during sequence blocks only with baclofen (i.e. baclofen – placebo)* 109
Fig. 4.3. ***Areas of the precuneus, cingulate gyrus and paracingulate gyrus showed different patterns of activity during early and late motor sequence learning between treatment conditions*** *(A; baclofen (S1 > S10:S13) > placebo (S1 > S10:S13))); COPE values for first sequence blocks (S1) and sequence blocks 10 to 13 (S10:13) (B)* 111

Fig. 4.4. ***Subcortical areas showed decreased activity during random blocks after baclofen administration compared to placebo*** *(A; baclofen (R1 & R2) < placebo (R1 & R2)); some cortical and cerebellar areas also showed decreased activity with baclofen after the application of the SRTT mean activation map as a pre-threshold mask (B)* 112
Fig. 4.5. ***Areas of increased learning-related activity (sequence > random) with baclofen*** *(baclofen (sequence > random) > placebo (sequence > random)), without a pre-threshold mask (A) and with the SRTT mean activation map as a pre-threshold mask (C). Corresponding COPE values in (B) and (D)* 113
Fig. 4.6. ***No significant effect of baclofen on a CANTAB computerised battery of working memory tests*** 116
Fig. 4.7. ***The effect of baclofen on mood components****: alertness (A), contentedness (B) and calmness (C). Columns and bars represent mean +/- SD.* 118
Fig. 5.1. ***Resting-state networks of interest: sensorimotor (A), visual (B) and default mode network (C).*** *These masks were then used as pre-threshold masks in resting-state group-level analyses* 138
Fig. 5.2. ***Baclofen significantly affected resting-state network connectivity strength****. Individual data points represent each participant's mean network connectivity strength. Columns and bars represent mean +/-SD. * p < 0.05 corrected for multiple comparisons* 139
Fig. 5.3. ***Areas with decreased connectivity in the sensorimotor network (A) and visual network (B)****. Colour scale represents significant p values from 0 to 0.05* 140
Fig. 5.4. ***Relationship between motor network strength and behavioural metrics****. (A) Motor network strength and response time during execute blocks in action-selection task; (B) Motor network strength and response time during random blocks in SRTT; (C) Motor network strength and SRTT learning metric* 142
Fig. 5.5. ***Example of GABA: Cre map in the sensorimotor network in one participant*** 143
Fig. 5.6. ***Pre-task [GABA] in the sensorimotor network****. (A) Sensorimotor pre-task [GABA] is not significantly different between treatment conditions; (B) No significant correlation between sensorimotor pre-task [GABA] and motor network strength; (C) No significant correlation between left M1 pre-task [GABA] and motor network strength; (D) No significant correlation between left premotor cortex pre-task [GABA] and motor network strength* 144
Fig. 6.1. ***Timeline of the testing session.*** *After the TMS set-up and first set of TMS measurements, the participant received the treatment (zolpidem or placebo) and then were familiarised with the tasks (action selection [AS], serial reaction time task [SRTT], temporal-order judgement and visuomotor adaptation [VMA]), which lasted approximately 15 minutes. At 1.6h post-treatment administration (PA), I collected another set of TMS measurements, after which participants completed the AS and the SRTT tasks. Then wIe collected another set of TMS measurements (2.35h PA), after which participants completed the VMA main task, the TOJ task and two working memory tests: the digit span and the auditory verbal learning test. I then collected the last set of TMS measurements (3.35h PA), after which participants completed the VMA washout task. Before the TMS measurements at each time point (baseline, 1.6h, 2.35h and 3.35h), we also asked the participants to fill in a mood questionnaire (MQ).* 159
*Fig. 6.2****. AS instructions and block order****. a) Sample EB instructions; b) Sample SB instructions* 160
Fig. 6.3. ***Serial reaction time task (SRTT)****. a) Rest screen; b) Sample cue for a random block; c) Block order* 162
*Fig. 6.4****. Visuomotor adaptation task (VMA).*** *a) Target circle: 8 potential targets (yellow) and cursor (red); b) Cursor trajectory in FB-ON blocks* 164
*Fig. 6.5****. Behaviour on Action Selection Task****. Both response times on execute blocks (A) and action selection times (B) were increased with zolpidem compared to placebo. **p<0.01* 174
*Fig. 6.6****. Behaviour on the Serial Reaction Time Task.*** *Response times during sequence learning were significantly slower after zolpidem administration compared to placebo (A). Learning RTs between treatment conditions on session 1 (B) and session 2 (C)* 178
*Fig. 6.7****. Behaviour on the visuomotor adaptation tasks: (A) main task, (B) washout task.*** *No significant difference in mean angular errors during feedback-on blocks (white background) and feedback-off blocks (grey background) between zolpidem and placebo session were detected on during the main task (A). Significantly worse retention with zolpidem was detected on the washout task (B)* 181
*Fig. 6.8****. Performance on the TOJ task****. Zolpidem significantly decreased the goodness-of-fit (A) and increased the JND (B) compared to placebo. * p < 0.05* 182
Fig. 6.9. ***The effect of zolpidem on TMS metrics.*** *Zolpidem did not significantly affect measurements of cortical excitability (rMT (A)), $GABA_A$-mediated inhibition (SICI 2.5ms (B); SICI 1ms: (C)) and $GABA_B$-mediated inhibition (LICI 150ms (D))* 184

Fig. 6.10. ***Performance on working memory tests****: digit span forwards (A) and backwards (B) and AVLT (C). Zolpidem significantly impaired performance on the AVLT but did not significantly affect performance on the digit span* _____ *186*
*Fig. 6.11****. The effect of zolpidem on mood components****. Zolpidem significantly decreased the participants' alertness (A), but not their contentedness (B) and calmness (C). There were no differences in the mood before treatment administration (D)* _____ *188*
Fig. 7.1. ***Putative changes in excitation-inhibition balance during stroke recovery*** _____ *209*
Fig. 7.2. ***Experimental design*** _____ *209*

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