

Physiology of neural networks in the dorsal raphe nucleus



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Abstract

5-HT (5-Hydroxy-tryptamine; serotonin) neurons located in the midbrain dorsal raphe nucleus (DRN) are implicated in many brain functions in health including aversive processing and are the target of the first line drug treatment for anxiety and depression, selective serotonin reuptake inhibitors (SSRIs). The research presented in this thesis employs silicon probe electrodes to investigate (1) the diversity of firing properties of both 5-HT and non-5-HT neurons in the DRN, (2) potential emergent population dynamics in the DRN network and (3) the effect on these parameters of an aversive foot-shock stimulus and treatment with the SSRI citalopram.

A first set of experiments established the silicon probe recording method. Histological analysis of electrode tracks and spike sorting of electrode signals confirmed the capacity of silicon probes to generate large-scale simultaneous recordings of DRN neurons. Through clustering of neuronal electrophysiological properties, three putative neuron types could be identified: slow regular firing neurons, fast firing neurons, and slow irregular firing neurons. The properties of these neurons were consistent with 5-HT, GABA, and generic non-5-HT neurons previously identified using cell labelling methods in the literature.

Next, the silicon probe recording method was applied to quantify the effects of an aversive foot-shock stimulus on the firing of DRN neurons. Widespread and diverse responses were observed among both putative 5-HT and non-5-HT neurons. Furthermore, analysis of the performance of stimulus decoders as a function of the number of DRN neurons used showed a positive relationship between decoder performance and neuron number, indicating DRN neurons may encode the foot-shock stimulus using a population code.

Silicon probe recordings were then applied to quantify interactions between simultaneously recorded neurons in the DRN network. Spike count correlations between pairs of putative 5-HT neurons were heterogeneous, with some neuron pairs being weakly correlated, others negatively correlated and yet others positively correlated. Groups of positively correlated putative 5-HT neurons formed neuronal ensembles. Spike count correlations between putative 5-HT and non-5-HT neurons were also heterogeneous: putative non-5-HT neurons could be positively correlated with putative 5-HT neurons, even being members of the same neuronal ensemble but, particularly for putative GABA neurons, were often negatively correlated with them when they were not.

In a final set of experiments, the effect on the DRN network of continued treatment with the SSRI citalopram was assessed. Citalopram treatment was associated with: (1) the generation of two populations of broad waveform putative 5-HT neurons with distinct firing patterns, consistent with citalopram having diverse effects on 5-HT neurons, (2) an attenuation of negative spike count correlations between putative 5-HT neurons, (3) an enhanced excitation of putative 5-HT neurons to aversive foot-shocks, (4) DRN putative GABA neurons having a reduced prevalence and lower firing rate than saline controls. Thus, these experiments revealed potential diverse effects of citalopram on 5-HT and non-5-HT DRN neurons and demonstrated that citalopram affects the sensitivity of putative 5-HT neurons to physiological stimuli.

These findings extend existing knowledge on 5-HT neuron diversity, the functioning of non-5-HT neurons within the DRN, and the ways by which 5-HT neurons function as part of the DRN network.

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List of abbreviations

5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
5-HTT	5-HT transporter
AP	Anterior/posterior
BNST	Bed nucleus of the stria terminalis
cAMP	Cyclic adenosine monophosphate
CeA	Central nucleus of the amygdala
DRN	Dorsal raphe nucleus
DV	Dorsal/ventral
GABA	Gamma-Aminobutyric Acid
LC	Locus coeruleus
LHA	Lateral hypothalamus
LHb	Lateral habenula
LSD	Lysergic acid diethylamide
ML	Medial/lateral
MAO	Monoamine oxidases
MRN	Median raphe nucleus
PAG	Periaqueductal gray
PFA	Paraformaldehyde
PFC	Prefrontal cortex
PVN	Paraventricular nucleus of the hypothalamus
SOM	Somatostatin-expressing interneuron
SSRI	Selective serotonin reuptake inhibitor
TPH	Tryptophan hydroxylase
vGLUT3	Vesicular Glutamate Transporter Type 3
VIP	Vasoactive intestinal peptide-expressing interneuron
VTA	Ventral tegmental area

Chapter 1: General introduction

1.1 The 5-HT system

The central 5-HT system and its 5-HT neurons located in the midbrain dorsal raphe nucleus (DRN) have attracted significant attention for their roles in brain function and implication in psychiatric disorders. This section will introduce some of the major findings that have contributed to the extensive research effort in this area and outline several fundamental properties of 5-HT neurons including mechanisms of synaptic transmission and anatomical connectivity.

1.1.1 Discovery of 5-HT

5-HT was independently discovered by two research groups in the 1930s and 1940s (Erspamer & Asero, 1952; Rapport et al., 1948; Whitaker-Azmitia, 1999). Initially identified as a vasoconstricting agent in the periphery, it was soon found to act as a neurotransmitter and subsequently localized in mammalian brain (Twarog, 1954; Twarog & Page, 1953).

Investigation into the relationship between 5-HT and brain function was initially driven by the structural similarities between 5-HT and the hallucinogen lysergic acid diethylamide (LSD; Woolley & Shaw, 1954). Notably, dysfunction in the central 5-HT system was hypothesized to underlie schizophrenia (Woolley & Shaw, 1954) and, in the 1950s, following an unsuccessful trial for the treatment of psychosis, the tricyclic compound imipramine was discovered to have antidepressant properties (Brown & Rosdolsky, 2015). Further research demonstrated that imipramine inhibited synaptic

reuptake of 5-HT, ultimately leading to the development of selective serotonin reuptake inhibitors (SSRIs) in the 1980s (Brown & Rosdolsky, 2015; López-Muñoz & Alamo, 2009). SSRIs remain the most commonly prescribed class of antidepressant and anxiolytic drugs to date.

The association between 5-HT and psychiatric disorders has contributed to a large research effort to uncover the role of 5-HT neurons in brain function. Although this work has not yet revealed a clear relationship between 5-HT and psychiatric disorders (Cowen & Browning, 2015; Moncrieff et al., 2022), work in this area has linked the 5-HT system to many brain functions in health and has uncovered many properties of 5-HT neurons.

1.1.2 5-HT synthesis and metabolism

5-HT is a member of the biogenic amines, a group of neuromodulators that also includes noradrenaline, dopamine, and histamine. 5-HT is synthesized from tryptophan in a two-step process: tryptophan hydroxylase first converts tryptophan to 5-hydroxytryptophan (5-HTP), which is then converted to 5-HT by L-amino acid decarboxylase (Bakshi & Tadi, 2023). The conversion of tryptophan to 5-HTP by tryptophan hydroxylase is rate-limiting. There are two isoforms of this enzyme, TPH1 and TPH2 with the latter found only in the brain in a population of 5-HT-producing neurons (Deguchi et al., 1972; Fitzpatrick, 1999; Koubi et al., 2001; Walther et al., 2003).

5-HT is packaged into vesicles by the vesicular monoamine transporter 2 (VMAT2), where it is stored until release (Erickson et al., 1996; Tamir & Gershon, 1990). 5-HT

can be released both at synapses and at axonal varicosities via volume transmission (Descarries et al., 2010; Fuxe et al., 2010).

Following its release from 5-HT neurons, 5-HT availability in the synapse is reduced by the 5-HT transporter, which is expressed both on 5-HT nerve terminals and cell bodies (Bakshi & Tadi, 2023; Bengel et al., 1997; Fujita et al., 1993). Cytosolic 5-HT is rapidly metabolized to 5-hydroxyindole acetic acid (5-HIAA) by the enzyme monoamine oxidase (MAO; Bakshi & Tadi, 2023; Bortolato et al., 2010). There are two isoforms of monoamine oxidase: MOA-A and MOA-B with MAO-B being more concentrated in 5-HT neurons (Grimsby & Wang, 1991; Levitt et al., 1982; Westlund et al., 1988).

1.1.3 5-HT receptors

To date fourteen 5-HT receptors have been discovered. They are grouped into seven families based on structural, signal transduction, and pharmacological similarities: 5-HT₁ to 5-HT₇. Within each family, there are receptor subtypes, such as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} receptors within the 5-HT₁ family (Bockaert et al., 2010; Sharp & Barnes, 2020).

All 5-HT receptors are G protein-coupled metabotropic receptors, with the exception of the 5-HT₃ receptor, which is a ligand-gated cation channel capable of fast excitatory transmission (Barnes et al., 2009).

There are both excitatory and inhibitory metabotropic 5-HT receptors. The inhibitory 5-HT_{1A} receptor and excitatory 5-HT_{2A/C} receptors are among the most abundantly expressed 5-HT receptor subtypes in the brain and best characterized. 5-HT_{1A} receptors are coupled to Gi proteins and can decrease neuronal excitability by (1)

inhibiting adenylyl cyclase to decrease concentrations of cAMP and (2) activating inward rectifying potassium channels (Masson et al., 2012; Montalbano et al., 2015; Sharp & Barnes, 2020). 5-HT_{2A/C} receptors are coupled to G_{q/11} proteins and their activation leads to increases in cellular concentrations of inositol trisphosphate and diacylglycerol, which can lead to increased postsynaptic excitatory currents *in vitro* (Marek & Marek, 2010; Sharp & Barnes, 2020).

5-HT receptors exhibit differential expression with respect to brain region and neuron type, resulting in a diversity of responses to 5-HT signaling (Koyama et al., 2017; Mengod et al., 2010; Palacios et al., 2016; Puig & Gullledge, 2011). Several 5-HT receptors including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{5B}, and 5-HT₇ receptors, are expressed by 5-HT neurons. 5-HT_{1A} receptors are expressed on the cell bodies and dendrites of 5-HT neurons and their activation reduces neuronal firing (Riad et al., 2000; Vandermaelen & Aghajanian, 1983; Vergé et al., 1985). 5-HT_{1B} receptors are trafficked to terminals where their activation reduces quantities of released neurotransmitter (Fink & Göthert, 2007; Vergé et al., 1985). These and other 5-HT receptors are also located postsynaptically on different neuron types (Mengod et al., 2010; Sharp & Barnes, 2020). For example, 5-HT_{2A} receptors are expressed on both pyramidal neurons and interneurons in the PFC (Puig & Gullledge, 2011).

1.1.4 Localization of 5-HT neurons

The localization of 5-HT neurons in mammalian brains was first visualized in the 1960s by Dahlström and Fuxe using a histological technique based on formaldehyde staining of monoaminergic cell bodies (Dahlström et al., 1964). They identified nine 5-HT cell clusters in the midbrain and brainstem, naming them B1 - B9, though these

are currently more commonly referred to as the raphe nuclei - 'raphe' being derived for the Latin for 'seam', noting their localization along the midline.

Subsequent studies have used histochemical analysis as well as retrograde and anterograde tracing techniques to investigate the neuron type composition and connectivity of the raphe nuclei (Dorocic et al., 2014; Huang et al., 2019; Michelsen et al., 2007, 2008; Xu et al., 2021). One notable finding from this work is that many of the anatomical features of the raphe nuclei appear to be conserved between humans and rodents, the latter being the primary model species that has been used in physiological investigations of the 5-HT system (Baker et al., 1990; Descarries et al., 1982; Ishimura et al., 1988; Steinbusch, 1981).

The raphe nuclei have been grouped into rostral and caudal groups based on anatomical properties and developmental lineage (Gaspar & Lillesaar, 2012; Jacobs & Azmitia, 1992). The rostral raphe nuclei are located in the midbrain and rostral pons and include the dorsal raphe nucleus (DRN), median raphe nucleus (MRN), and caudal linear nucleus. These nuclei send the majority of ascending 5-HT projections to the forebrain. Among the rostral raphe nuclei, the DRN contains the largest number of 5-HT neurons. The DRN has been estimated to contain the cell bodies of 9000 5-HT neurons in mice, approximately 50% of the total number of 5-HT neurons in the raphe nuclei (Ishimura et al., 1988). The caudal raphe nuclei include the raphe pallidus, raphe obscurus and raphe magnus. 5-HT neurons located in these nuclei send the majority of descending projections to the brain stem and spinal cord.

1.1.5 Neurochemical diversity of the DRN

The DRN does not exclusively contain 5-HT neurons. Estimates of the proportion of DRN neurons that utilize 5-HT have ranged from as high as 70% in humans and cats to as low as 33% in rats (Baker et al., 1991; Descarries et al., 1982; Wiklund et al., 1981). It is currently unclear whether this discrepancy reflects interspecies differences or measurement error as different methodologies have been used to generate these estimates: formaldehyde-based staining in humans, immunohistochemistry in cats, and uptake of tritiated 5-HT in rats.

The neurochemical identity of non-5-HT DRN neurons has been investigated using histological methods such as immunohistochemistry and autoradiographic labelling (Michelsen et al., 2008). These studies have identified populations of glutamate, GABA, dopamine, nitric oxide, peptide, and noradrenaline neurons (Belin et al., 1979; Gamrani et al., 1979; Grzanna & Molliver, 1980; Kaneko et al., 1990; Ochi & Shimizu, 1978). Neuron types can be concentrated in subregions of the DRN (Allers & Sharp, 2003; Calizo et al., 2011; Huang et al., 2019; Xu et al., 2021). For example, GABA neurons have been reported to be most abundant in the lateral wings, glutamate neurons rostrally, and 5-HT neurons ventromedially (Allers & Sharp, 2003; Calizo et al., 2011; Xu et al., 2021).

Several neurotransmitters, including glutamate, GABA, nitric oxide, and several neuropeptides have been found to be co-localized in 5-HT neurons (Gagnon & Parent, 2014; Huang et al., 2019; Okaty et al., 2019; Ren et al., 2019). Data from in vitro electrophysiological recordings indicates that 5-HT neurons may release these messengers as co-transmitters under certain conditions. There is strongest evidence for co-transmission of glutamate. For example, pharmacological analysis of

postsynaptic potentials evoked from optogenetic activation of DRN 5-HT terminals in basolateral amygdala slices indicated that these neurons released glutamate at low- (~1 Hz) and 5-HT at high- (~20 Hz) frequencies of optogenetic stimulation (Sengupta et al., 2017).

1.1.6 Efferent connectivity of DRN neurons

Neuroanatomical tracing approaches have mapped the projections of DRN 5-HT neurons and found that they project to nearly all forebrain regions as well as midbrain and brainstem nuclei such as the locus coeruleus (LC), substantia nigra, and ventral tegmental area (VTA; Azmitia & Segal, 1978; Bang et al., 2012; Fernandez et al., 2016; Michelsen et al., 2008; Petrov et al., 1994; Waselus et al., 2011). Ascending 5-HT projections travel through three main pathways: dorsal, medial, and ventral (Michelsen et al., 2007). The dorsal ascending pathway projects to the striatum and globus pallidus. The medial ascending pathway mainly targets the substantia nigra and caudate putamen. The ventral ascending pathway, which also contains fibers from other raphe nuclei, projects to various forebrain regions, including the hippocampus, thalamus, habenula, septum, amygdala, and cortex.

Both 5-HT and GABA neurons send local projections within the DRN (Adell et al., 2002; Allers & Sharp, 2003; Q. P. Wang et al., 1992; Weissbourd et al., 2014). 5-HT neurons can project directly onto neighboring 5-HT and GABA neurons, though axonal varicosities have also been identified on 5-HT fibers within the DRN, suggesting that these neurons may additionally signal locally via volume transmission (Adell et al., 2002; Weissbourd et al., 2014). DRN GABA neurons form synapses with local 5-HT and GABA neurons and there is evidence that they may do so differentially depending on their spatial localization within the nucleus (Weissbourd et al., 2014).

Non-5-HT neurons can also send projections out of the DRN (Aznar et al., 2004; Bang & Commons, 2012; Halberstadt & Balaban, 2006; McDevitt et al., 2014; Petrov et al., 1994; Xu et al., 2021). For example, recent whole brain mapping of the projections of DRN glutamate and GABA neurons identified the primary targets of these neurons as subcortical regions such as the VTA, MRN, lateral hypothalamic area (LHA), bed nucleus of the stria terminalis (BNST) and amygdalar nuclei (Xu et al., 2021).

1.1.7 Afferent connectivity of DRN neurons

Whole brain mapping of inputs to DRN 5-HT neurons has revealed widespread input from numerous cortical regions and subcortical nuclei (Dorocic et al., 2014). Notably, 5-HT neurons were found to receive dense input from brain regions associated with emotional processing, such as the central nucleus of the amygdala (CeA), BNST, periaqueductal gray (PAG), and prefrontal cortex (PFC; Dorocic et al., 2014). In addition, 5-HT neurons receive dense inputs from subcortical and brain stem structures associated with autonomic and sensory functions, such as the nucleus raphe magnus which is implicated in nociception (Dickenson et al., 1979; Sakai et al., 1977; Q. P. Wang & Nakai, 1994).

GABA DRN neurons have been proposed to distribute input onto 5-HT neurons: these neurons receive excitatory input from the PFC and lateral habenula (LHb) and electrical stimulation of these brain regions can elicit short-latency activation of putative GABA neurons and longer latency inhibition of putative 5-HT neurons that are dependent on GABA receptors (Hajós et al., 1998; Varga et al., 2001, 2003). Recent work has also implicated glutamate neurons in processing afferent input to the DRN. These neurons were revealed to have highly correlated inputs with DRN

GABA neurons (Xu et al., 2021), potentially representing divergent pathways for the modulation of DRN 5-HT neurons.

1.1.8 Diversity of gene expression profiles among DRN neurons

Several studies have used single cell RNA sequencing technology to quantify the gene expression profiles of raphe 5-HT neurons (Huang et al., 2019; Niederkofler et al., 2016; Okaty et al., 2015; Ren et al., 2019; Spaethling et al., 2014). The findings of these studies indicate that there are several discrete subpopulations of 5-HT neurons that express different complements of genes and have different patterns of anatomical connectivity.

One recent study applied high throughput technology to analyze the gene expression profiles of 999 5-HT neurons, identifying 7 clusters within the DRN (Ren et al., 2019). One way by which these clusters differed was by the expression of neurotransmitter receptors: subpopulations of 5-HT neurons had differing expression of 5-HT receptors including 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{5B} as well as receptors to other neurotransmitters and neuropeptides including alpha adrenoceptors and neuropeptide Y receptors. Another notable difference was the expression of markers for co-transmitters: subpopulations of 5-HT neurons expressed the vesicular transporter vGLUT3, consistent with co-transmission of glutamate, while others did not. Finally, it was also reported that 5-HT neurons with similar gene expression profiles were often located close together within the DRN and had similar projection targets (Ren et al., 2019). This latter link between gene expression, localization within the DRN, and anatomical connectivity has also been reported by several similar studies (Huang et al., 2019; Okaty et al., 2019).

Single cell RNA sequencing has been applied to investigate the gene expression profiles of non-5-HT neurons within the DRN. One recent study applied high throughput technology to reveal the expression profile of 2041 DRN neurons including 5-HT, glutamate, GABA, dopamine, and peptide neurons (Huang et al., 2019). One way by which 5-HT and non-5-HT neurons differed was according to the expression of 5-HT receptors. The majority of 5-HT neurons expressed 5-HT_{1A} receptors and most non-5-HT neurons did not, consistent with previous findings from histological studies (Sotelo et al., 1990). Conversely, 5-HT neurons had low expression of 5-HT_{2C} receptors, but these receptors were widely expressed on GABA, glutamate, and peptide neurons. Notably, diversity was identified among non-5-HT neurons, with three subpopulations of GABA and five subpopulations of glutamate neurons having distinct gene expression profiles (Huang et al., 2019).

1.2 Electrophysiological investigations of the dynamics of DRN neurons

In vivo electrophysiological recordings have been one of the primary methodologies used to investigate the role of 5-HT neurons in brain function. This method enables the dynamics of single neurons to be monitored (Buzsáki, 2004) and, as is discussed below, has been applied in electrophysiological studies of the DRN to quantify relationships between neuronal activity and cognitive and behavioral variables.

1.2.1 Electrophysiological characteristics of DRN neurons

Three electrophysiological profiles have commonly been reported in the rodent DRN: (1) slow and regular spiking neurons with broad spike waveforms, (2) slow and irregular spiking neurons without broad spike waveforms, and (3) fast-spiking

neurons with narrow spike waveforms (Aghajanian et al., 1978; Allers & Sharp, 2003; Nakahama et al., 1981; Sakai, 2011; Schweimer et al., 2011; Schweimer & Ungless, 2010; Varga et al., 2001, 2003). Studies have investigated the neurochemical identity of neurons with these electrophysiological profiles using intracellular and juxtacellular labelling techniques followed by immunocytochemical analysis of the labeled neurons (Aghajanian & Vandermaelen, 1982a; Allers & Sharp, 2003; Schweimer et al., 2011; Schweimer & Ungless, 2010; Vandermaelen & Aghajanian, 1983).

Studies that have used intracellular labelling techniques in DRN slices have reported that most slow and regular spiking neurons were immunopositive for 5-HT markers whereas slow irregular and fast spiking neurons were not, consistent with these neuronal populations being mostly 5-HT and non-5-HT neurons, respectively (Aghajanian & Vandermaelen, 1982a, 1982b). Similar results have been obtained more recently *in vivo* using juxtacellular labelling (Allers & Sharp, 2003; Hajós et al., 2007; Schweimer et al., 2011; Schweimer & Ungless, 2010). For example, one study reported that slow and regular firing DRN neurons were immunopositive for TPH2/5-HT, whereas slow irregular firing DRN neurons were TPH2/5-HT immunonegative (Allers & Sharp, 2003). Finally, there is also *in vivo* juxtacellular evidence that fast spiking DRN neurons are mostly GABA neurons. In particular, it was reported in one study that that most (8/10) neurons with a fast-spiking profile were immunopositive for GAD, a marker of GABA neurons (Allers & Sharp, 2003).

Although *in vivo* juxtacellular studies strongly support a link between electrophysiological characteristics and neurotransmitter identity of DRN neurons, these studies have also identified diversity in the properties of 5-HT and non-5-HT

neurons (Allers & Sharp, 2003; Kocsis et al., 2006; Schweimer & Ungless, 2010). For example, these studies have reported the presence of fast firing DRN neurons that were immunopositive for 5-HT as well as some DRN neurons with a slow and regular profile that were immunonegative for both 5-HT and TPH2 (Allers & Sharp, 2003; Kocsis et al., 2006; Schweimer & Ungless, 2010). It should be pointed out, however, that immunonegativity in such studies should be interpreted with caution as antigen expression may be below the level of detection.

1.2.2 Methods for estimating the neurochemical identity of DRN neurons

The electrophysiological characteristics of DRN neurons have been used widely to estimate the neurochemical identity of recorded neurons. The most common approach has been to classify those neurons with a slow and regular firing pattern with broad spike waveforms as putative 5-HT neurons and those with a slow and irregular spiking profile as putative non-5-HT neurons (Aghajanian et al., 1978; Fornal et al., 1996; Heym et al., 1982; Jacobs & Azmitia, 1992; Sakai & Crochet, 2001; Waterhouse et al., 2004). Several studies have additionally classified fast spiking neurons with narrow waveforms as putative GABA neurons (Hajós et al., 1998; Varga et al., 2001, 2003). However, as noted above, variation in electrophysiological properties identified in juxtacellular-labelling studies indicates that this approach may lead to both false positive and false negative misclassifications.

Many studies have additionally taken to identifying DRN neurons through their responsivity to 5-HT_{1A} agonists or agents which indirectly increase concentrations of 5-HT in the DRN, such as SSRIs (Fornal et al., 1996; Jacobs & Azmitia, 1992; Sakai & Crochet, 2001; Waterhouse et al., 2004). The idea is that 5-HT neurons should be

differentially inhibited by these drugs due to their high expression of 5-HT_{1A} autoreceptors. Selective expression of 5-HT_{1A} receptors by 5-HT neurons is supported by both histological and RNA sequencing studies (Huang et al., 2019; Sotelo et al., 1990). Moreover, selective inhibition, at least among spontaneously active neurons, is supported by a slice electrophysiological study that employed intracellular labelling (Kirby et al., 2003). However, variation in 5-HT_{1A} receptor expression has been reported for both 5-HT and non-5-HT DRN neurons (Kirby et al., 2003; Marinelli et al., 2004; Ren et al., 2019), indicating that estimates of neurochemical identity using this approach have the potential for some misclassifications.

Several recent studies have applied optogenetic tagging (optotagging) to identify 5-HT neurons (Cohen et al., 2015; Li et al., 2016a; Z. Liu et al., 2014). This approach involves targeting a light-sensitive cation channel to 5-HT neurons and then implanting both a recording electrode and an optic fiber so that 5-HT neurons can be identified by their short-latency responses to light application. This approach is likely to have a low false positive rate but may suffer from a higher false negative rate, as the yield of ‘tagged’ neurons is typically low, possibly due to variable levels of opsin expression.

1.2.3 Neural systems controlling DRN dynamics

There has been a large research effort to identify the neural systems that control the dynamics of DRN neurons and especially 5-HT neurons. The findings of this work indicates that 5-HT neurons are under control from both local neurons in the DRN as well as external brain regions (Sharp et al., 2007).

Several mechanisms have been identified by which the activity of 5-HT neurons could be modulated by neighboring neurons in the DRN network. As outlined previously, neuroanatomical studies have identified projections both among and between 5-HT and GABA neurons within the DRN (Adell et al., 2002; Q. P. Wang et al., 1992; Weissbourd et al., 2014). Notably, these studies have reported associations between spatial localization within the DRN and local connectivity. 5-HT neurons had most dense local inputs from GABA neurons located laterally and from other 5-HT neurons located ventrally (Weissbourd et al., 2014).

Pharmacological studies have further demonstrated that systemic administration of agonists of a variety of 5-HT receptors can modulate 5-HT neuron firing (Sharp et al., 2007). For example, inhibition of putative 5-HT neurons was reported following administration of 5-HT_{1A} and 5-HT_{2C} receptor agonists and excitation of these neurons has been reported following administration of 5-HT₄ and 5-HT₆ receptor agonists (Boothman et al., 2006; Brouard et al., 2015; R. Liu et al., 2000; Lucas & Debonnel, 2002; Sprouse & Aghajanian, 1987).

It is likely that many interactions between 5-HT neurons involve intermediary neurons (Sharp et al., 2007). For example, although putative 5-HT neurons are inhibited by 5-HT_{2C} receptor agonists, RNA sequencing and histological studies indicate that most 5-HT neurons do not express these receptors (Cornea-Hébert et al., 1999; Huang et al., 2019). Instead, this inhibition is likely mediated, at least in part, by local GABA neurons. One study reported that 5-HT_{2C} receptor agonists increased expression of c-fos in DRN GABA neurons, and inhibitions of putative 5-HT neurons that were reversed by GABA-A receptor blockade (Boothman et al., 2006). Local control of 5-HT neurons may also be mediated by neurons in external

brain structures, such as the PFC, which have reciprocal connectivity with the DRN (Sharp et al., 2007).

Many brain regions have been implicated in the control of DRN neuron dynamics including neuromodulatory nuclei such as the locus coeruleus, limbic structures such as the CeA as well as brain stem nuclei such as the nucleus raphe magnus (Dorocic et al., 2014; Svensson et al., 1975; Weissbourd et al., 2014). The PFC and LHb may be particularly important regulators of DRN dynamics. Neurons in these brain regions send dense projections to the DRN and target 5-HT, GABA and glutamate neurons (Aghajanian & Wang, 1977; Dorocic et al., 2014; Gervasoni et al., 2000; Peyron et al., 1998; Sego et al., 2014; Varga et al., 2001; Weissbourd et al., 2014; Xu et al., 2021). As mentioned previously, activation of PFC and LHb neurons can modulate both putative GABA and 5-HT neurons within the DRN, doing so in a manner consistent with GABA neurons distributing an inhibitory signal onto 5-HT neurons (Hajós et al., 1998; Varga et al., 2001, 2003; M. R. Warden et al., 2012).

Data from electrophysiological studies also indicates that input from the PFC and LHb may impact 5-HT and non-5-HT neurons in the DRN in different ways. For example, in one study that recorded from both putative 5-HT and non-5-HT neurons while electrically stimulating the PFC and LHb, ~40 - 50% of putative GABA neurons were excited by PFC and LHb stimulation. Similar diversity in responses was observed for putative 5-HT neurons (Varga et al., 2003). This idea of differential control is supported by recent neuroanatomical studies that have reported that input from both the PFC and LHb targets specific DRN subregions and specific neuron types (Sego et al., 2014; Weissbourd et al., 2014). Neuroanatomical studies have also identified both

excitatory and inhibitory pathways from these nuclei to the DRN (Jankowski & Sesack, 2004; Sego et al., 2014; Weissbourd et al., 2014).

1.2.4 Features of DRN neuron dynamics

Many studies have monitored the activity of DRN neurons in awake animals and reported correlations between neuronal firing and behavioral and cognitive variables. Although it is beyond the scope of this text to thoroughly review this literature, it is informative to outline some properties of DRN neuron dynamics that have been frequently reported in such studies.

An association between the firing of DRN neurons and numerous cognitive and behavioral variables have been reported. These range from (1) autonomic states like sleep, wakefulness and behavioral arousal, as well as bodily functions such as temperature regulation, (Oikonomou et al., 2019; Sakai, 2011; Schneeberger et al., 2019), (2) sensory stimuli such as odors and mild aversive foot-shocks (Ranade & Mainen, 2009; Schweimer & Ungless, 2010), (3) motor outputs such as repetitive buccal movements and locomotion (Fornal et al., 1996; Jacobs & Fornal, 1997, 1999), as well as (4) cognitive variables such as stimuli conditioned to predict reward and punishments (Cohen et al., 2015; Dayan & Huys, 2009; Nakamura, 2013). Ranade and Mainen explicitly investigated the plurality of variables tracked by DRN neurons (Ranade & Mainen, 2009). They recorded from DRN neurons during an odor guided decision-making task and quantified associations between neuronal firing and a large set of behaviors ranging from locomotion, odor stimuli, to reward state. For all behaviors measured, there was at least one DRN neuron that showed significant tuning to it. Moreover, individual neurons had tuning to multiple behavioral variables (e.g., increased spike rate during locomotion and following the presentation of a

specific odor). This latter observation of mixed selectivity has also been reported by more recent studies (Hayashi et al., 2015; Paquelet et al., 2022).

Although most electrophysiological studies of the DRN have reported solely on the properties of putative 5-HT neurons, the findings of those that have reported on the functional properties of non-5-HT neurons indicates that their firing also tracks cognitive and behavioral variables (Aghajanian & Wang, 1977; Ranade & Mainen, 2009; Sakai, 2011; Urbain et al., 2006). For example, non-5-HT neurons identified using juxtacellular labelling had altered firing during different stages of the sleep-wake cycle (Sakai, 2011). The importance of non-5-HT neurons has been highlighted by several recent studies that have used optogenetic manipulations in mice to demonstrate the capacity of these neurons to alter behavioral outputs (Challis et al., 2013; Cho et al., 2017; Qi et al., 2014; Schneeberger et al., 2019). For example, optogenetic activation of DRN dopamine neurons altered time spent asleep while activation of DRN glutamate neurons altered reward seeking behaviors (Cho et al., 2017; Qi et al., 2014).

DRN neurons can track behavioral and cognitive variables over multiple timescales, with alterations in firing on timescales ranging from milliseconds to hours. For example, DRN neurons have been reported to have altered tonic firing during different stages of the sleep wake cycle, which varied on timescales of minutes and hours (Sakai, 2011), whereas following the presentation of sensory stimuli changes in spiking activity was reported at latencies as short as 50 msec (Ranade & Mainen, 2009). The signaling of DRN neurons over multiple timescales was also investigated in one recent study by Cohen et al (Cohen et al., 2015) in which optotagged 5-HT neurons in the mouse DRN were monitored during a complex behavioral task that

tracked behavioral variables that varied over multiple temporal scales. On slow timescales, mice performed blocks of repeated punished or rewarded trials whereas on fast timescales, mice processed several emotionally salient stimuli within a trial (e.g., a reward or a stimulus conditioned to predict a reward). Subpopulations of 5-HT neurons were identified that were tuned to stimuli on both fast and slow timescales. Cohen et al speculated that the slower timescale tonic dynamics may track the emotional valence of environments while phasic deviations in firing may signal the presence of specific stimuli (Cohen et al., 2015). This idea of temporal multiplexing within DRN neuron signaling has been proposed for other behavioral variables including aversion and arousal (Hayashi et al., 2015; Jacobs & Azmitia, 1992).

Most studies that have reported on the modulation of 5-HT neuron dynamics during behavior have identified variation among neurons (Cohen et al., 2015; Kocsis et al., 2006; Schweimer et al., 2011; Schweimer & Ungless, 2010). For example, following the presentation of a sucrose reward, some subpopulations of optotagged 5-HT neurons had increased firing, while the firing of others was decreased and that of others still was unaffected (Cohen et al., 2015). Similarly, measurements of putative DRN 5-HT neuron firing have identified subpopulations with opposing tuning to states of sleep and wakefulness. Thus, single unit recordings in mice identified one large subpopulation of putative 5-HT DRN neurons that had higher firing during wakefulness than NREM sleep and another smaller subpopulation with lower firing during wakefulness than NREM sleep (Sakai, 2011; Sakai & Crochet, 2001). Divergent responses among putative 5-HT neurons have also been reported with respect to aversive stimuli (Cohen et al., 2015; Hayashi et al., 2015; Schweimer & Ungless, 2010) and motor behaviors (Fornal et al., 1996; Ranade & Mainen, 2009).

1.2.5 Differentiated versus unified 5-HT signaling

Several previous theories posited that DRN 5-HT neurons send a unified (global) signal (Deakin & Graeff, 1991; Jacobs & Azmitia, 1992). Support for this idea came from the neurobiological properties of 5-HT neurons such as their highly collateralized projections (Descarries et al., 1982; Fallon & Loughlin, 1982; Imai et al., 1986) and capacity to engage in volume transmission (Fuxe et al., 2010), as well as from the findings of early electrophysiological studies that indicated that these neurons had relatively homogeneous pharmacological and functional properties. For example, early electrophysiological recordings in cats indicated that most putative 5-HT neurons altered their firing in the same way during the sleep wake cycle (Trulsson & Jacobs, 1979) and recordings in rodents indicated that presumed 5-HT neurons in the DRN were collectively inhibited by the hallucinogen LSD compared to neurons in surrounding brain regions (Aghajanian et al., 1968; Jacobs & Azmitia, 1992).

Many recent theories of 5-HT signaling have instead hypothesized that the population of 5-HT neurons in the DRN is comprised of severally functionally segregated submodules (Abrams et al., 2004; Calizo et al., 2011; Commons, 2015; Gaspar & Lillesaar, 2012; Hale & Lowry, 2011; Huang et al., 2019; Okaty et al., 2015, 2019; Ren et al., 2018, 2019; Weissbourd et al., 2014). Such 5-HT submodules have been proposed to segregate according to several factors including developmental cell lineage (Gaspar & Lillesaar, 2012; Okaty et al., 2015), gene expression profile (Huang et al., 2019; Niederkofler et al., 2016; Ren et al., 2019), localization within the DRN (Calizo et al., 2011; Hale & Lowry, 2011) and anatomical connectivity (Commons, 2015; Weissbourd et al., 2014).

A common theme in these ideas is that submodules of 5-HT neurons may have differential dynamics resulting from differential afferent inputs (Abrams et al., 2004; Commons, 2015; Weissbourd et al., 2014) or sensitivity to inputs through expression of neurotransmitter receptors (Abrams et al., 2004; Niederkofler et al., 2016). Submodules may then affect brain function in different ways through differential projections (Abrams et al., 2004; Ren et al., 2018, 2019; Waselus et al., 2011) or release of 5-HT and co-transmitters (Huang et al., 2019; Prouty et al., 2017; Ren et al., 2018, 2019; Weissbourd et al., 2014).

Evidence supporting such a ‘differentiated 5-HT system’ has come from several recent studies that combined 5-HT neuron segmentation with functional manipulations of specific subpopulations (Niederkofler et al., 2016; Ren et al., 2018). Thus, in one recent study in mice Niederkofler et al identified two subpopulations of 5-HT neurons with differential expression of dopamine receptors (Niederkofler et al., 2016). Although small in number, inhibition of these subpopulations was found to modulate aggressive behavioral outputs to a greater extent than other 5-HT neurons. In another study (Ren et al., 2018), Ren et al reported that amygdala-projecting and PFC-projecting 5-HT neurons had divergent activity, as indicated by bulk fiber photometric measurements, during forced swim and elevated plus maze tasks which are associated with depressive and anxiety like behaviors in rodents, respectively. Chemogenetic inhibition of amygdala-projecting 5-HT neurons decreased behaviors associated with anxiety and depression on these tasks while inhibition of PFC projecting neurons had the opposite effect.

Another way by which it might be possible to detect differentiated 5-HT neuron signaling is via the analysis of network dynamics (Humphries, 2018). If 5-HT neurons

form segregated submodules, their dynamics may be coordinated into intercorrelated neuronal ensembles, which have been frequently reported in other brain regions including the hippocampus and cortex, and recently in noradrenergic neurons of the LC (Carillo-Reid & Yuste, 2020; Humphries, 2011; Kodama et al., 2018; Miller et al., 2014; Totah, 2018). One recent study employed one photon calcium imaging to monitor the simultaneous dynamics of groups of 5-HT neurons in the DRN (Paquelet et al., 2022). It was found that neuronal correlations had a modular structure, with 5-HT neurons forming neuronal ensembles.

This preliminary evidence of 5-HT neuronal ensembles in the DRN further supports the idea that 5-HT neurons use a differentiated code. However, many properties of 5-HT ensembles remain poorly understood. For example, it is not known whether the neuronal composition of ensembles is static (e.g., being genetically or developmentally defined neuron types) or changes dynamically over time. Nor is it known how the dynamics of 5-HT ensembles relates to the activity of other neurons in the DRN network, such as local GABA neurons. Notably, network dynamics within the DRN such as neuronal ensembles have, to our knowledge, not yet been identified using electrophysiological methods.

1.2.6 Effects of aversive stimuli on the dynamics of DRN neurons

DRN 5-HT neurons are implicated in the regulation of emotionality and especially negative emotional states (Dayan & Huys, 2009). A primary approach that has been employed to investigate the role of 5-HT neurons in aversive processing is to measure the effect of aversive stimuli, such as mild foot-shocks or air puffs to the eye, on the dynamics of 5-HT neurons in the DRN. The findings of early electrophysiological studies using this approach were mixed. Although one early in vivo study in

anesthetized rodents reported that DRN neurons were tuned to aversive peripheral nerve stimulation (Aghajanian et al., 1978), subsequent studies in behaving cats reported that putative 5-HT neurons were mostly insensitive to stressors (Jacobs & Fornal, 1999; Wilkinson & Jacobs, 1988) and another in rodents found that, while these neurons were responsive to aversive stimuli, responses were highly heterogeneous and of low-magnitude (Montagne-Clavel et al., 1995).

Stronger evidence for DRN 5-HT neuron dynamics being modulated by aversive stimuli came from studies that measured 5-HT release and c-fos expression. Several studies reported that electric shocks and other stressors increased c-fos expression in the DRN including among 5-HT neurons (Grahn et al., 1999; Marcinkiewicz et al., 2016; Takase et al., 2004). In microdialysis studies, aversive stimuli and stressors were reported to increase the release of 5-HT both in the DRN and in various forebrain regions including the amygdala and PFC (Kawahara et al., 1993; Maswood et al., 1998; Yoshioka et al., 1995). This idea of aversive stimuli triggering activation of 5-HT neurons was later confirmed by electrophysiological recordings of DRN neurons in monkeys and rodents, including 5-HT neurons identified using juxtacellular labelling and optogenetic tagging (Cohen et al., 2015; Hayashi et al., 2015; Schweimer & Ungless, 2010).

There has been a research effort to identify the brain regions that drive and regulate activity in the DRN during aversive processing. Sources of excitatory drive likely include (1) projections from subcortical and brain stem nuclei such as the paraventricular thalamus and nucleus raphe magnus which are associated with nociception (Dickenson et al., 1979; Q. P. Wang & Nakai, 1994) and (2) the LHb which is associated with aversion and the processing of negative emotional states (Lazaridis

et al., 2019; Lecca et al., 2014; Matsumoto et al., 2007). In respect to the latter, optogenetic inhibition of glutamatergic LHB neurons that project to the DRN attenuated the behavioral effects of electric tail-shocks on behavior and associated increases in 5-HT release in the amygdala (Dolzani et al., 2016).

Another potential inhibitory pathway involves top-down control of the DRN by the PFC, which is implicated in modulating other sensory and emotional processes (Arnsten, 2009; Paneri & Gregoriou, 2017). PFC neurons have been proposed to inhibit aversive processing by DRN 5-HT neurons in behavioral contexts when it is possible to control the presentation of aversive stimuli (Amat et al., 2005; Maier, 2015; Rozeske et al., 2011). Thus, uncontrollable stress was associated with a greater quantity of extracellular 5-HT in the DRN than controllable stress, but not when the PFC was inactivated (Amat et al., 2005). Similarly, optogenetic activation of DRN-projecting PFC neurons reduced immobility on a forced swim task (M. R. Warden et al., 2012).

Multiple stimuli and cognitive variables related to negative emotional states, stress, and aversion can modulate the dynamics of DRN neurons. These include (1) the processing of both conditioned and unconditioned aversive stimuli such as foot-shocks and air puffs to the eye (Cohen et al., 2015; Hayashi et al., 2015; Schweimer & Ungless, 2010; Sengupta et al., 2017), (2) the rate of punishments in the current environment (Cohen et al., 2015; Hayashi et al., 2015), (3) the acquisition, and expression of conditioned fear (Marcinkiewicz et al., 2016; Sengupta et al., 2017), (4) behavioral tasks associated with anxiety-like behaviors such as the elevated plus maze (Marcinkiewicz et al., 2016; Ren et al., 2018), and (5) behavioral tasks associated with depressive-like behaviors such as forced swimming and social defeat (Challis et al.,

2013; Kirby et al., 2007; Ren et al., 2018). Notably, the dynamics of DRN neurons, including both 5-HT and non-5-HT neurons, can additionally be modulated by stimuli and cognitive variables with a positive emotional valance (Cohen et al., 2015; Hayashi et al., 2015; Li et al., 2016a; Z. Liu et al., 2014; Nakamura, 2013; Qi et al., 2014). For example, these neurons have been reported to be tuned to conditioned and unconditioned sucrose rewards and environmental rates of rewards (Cohen et al., 2015; Hayashi et al., 2015).

It is not yet established how such diverse cognitive and behavioral variables are encoded in the dynamics of DRN neurons. One possibility is that different subpopulations of neurons respond to different variables. Consistent with this idea, aversive stimuli appear to selectively activate subsets of DRN 5-HT neurons, with some evidence suggesting that neurons are differentially activated depending on their projection targets. Thus, histological studies have reported that subregions of the DRN are differentially enriched in c-fos immunoreactive neurons following the presentation of aversive stimuli and stressors (Grahn et al., 1999; Kirby et al., 2007; Marcinkiewicz et al., 2016; Takase et al., 2004). Electrophysiological studies have additionally identified distinct 5-HT neurons that were activated, inhibited and non-responsive to aversive foot-shocks (Cohen et al., 2015; Schweimer & Ungless, 2010).

To investigate the association between responsiveness to aversive stimuli and anatomical connectivity, one study combined c-fos immunolabeling with retrograde tracing to demonstrate that 5-HT neurons projecting to the BNST were preferentially activated by conditioned electric shocks (Marcinkiewicz et al., 2016). Additionally, a microdialysis study demonstrated that forced swim was associated with differential effects on 5-HT release according to brain region: extracellular 5-HT increased in the

striatum and decreased in the lateral septum (Kirby et al., 1995). Microdialysis studies have also compared the effects of different stressors on 5-HT release in different forebrain regions and found evidence of regional specific changes (Adell et al., 1997; Kirby et al., 1997).

Another explanation for how multiple cognitive and behavioral variables related to emotionality could be encoded in the dynamics of DRN neurons could be temporal multiplexing. Thus, a single neuron could encode one variable using its tonic firing rate while other variables are encoded by phasic deviations from it. This idea was proposed by Hayashi et al, to account for the observation that many electrophysiologically recorded DRN neurons in monkeys were tuned to rewards and punishments on different timescales (Hayashi et al., 2015). In the latter study, many neurons tracked slow timescale aversive variables such as the probability of receiving a punishment during a set of trials and these neurons tracked variables associated with reward on fast timescales, such as having phasic increases in spiking following the presentation of conditioned and unconditioned rewards (Hayashi et al., 2015). However, an optotagging study in mice identified DRN 5-HT neurons that were tuned to both reward- and aversion-associated stimuli on both fast and slow timescales (Cohen et al., 2015).

The role of non-5-HT DRN neurons in aversive processing is not clear. The anatomical connectivity of these neurons is consistent with them acting either as interneurons, potentially feeding input onto 5-HT neurons, or as principal neurons, effecting change at downstream projection sites. As mentioned in previous sections, both DRN glutamate and GABA neurons receive afferent input from regions associated with nociception and aversion as well as from regions associated with the regulation of

emotionality (Weissbourd et al., 2014; Xu et al., 2021). These neurons in turn are thought to project locally onto 5-HT neurons as well as externally to regions including the VTA, LHb, CeA, and PFC (Weissbourd et al., 2014; Xu et al., 2021).

Two early electrophysiological studies reported that some putative non-5-HT DRN neurons were differentially activated by aversive stimuli, whilst putative 5-HT neurons were either inhibited or not responsive (Aghajanian et al., 1978; Nakahama et al., 1981). Another study reported that, following the presentation of an aversive stimulus, c-fos expression in the DRN occurred predominantly (~ 85%) in GABA neurons with only a small fraction (~ 5%) of c-fos immunopositive neurons being 5-HT neurons (Roche et al., 2003). One recent study employed fiber photometry to measure the effect of aversive stimuli on the bulk activity of DRN GABA neurons, comparing their responsivity to optotagged 5-HT neurons (Li et al., 2016a). In the latter study, 5-HT neurons were generally unresponsive to aversive foot-shocks and GABA signals were activated by these stimuli. The potential importance of GABA neurons in the regulation of negative emotional states by the DRN was highlighted by a recent study that demonstrated that the acquisition of depressive-like behaviors following repeated social defeat could be prevented by optogenetically inhibiting these neurons (Challis et al., 2013). However, many recent electrophysiological studies have either focused recordings to 5-HT neurons or not separated DRN neurons according to their neurochemical identity (Cohen et al., 2015; Hayashi et al., 2015).

1.2.7 Effects of SSRIs on DRN dynamics.

SSRIs are an important class of antidepressant medication and are currently the first line drug treatment for both depression and several anxiety disorders in the UK

(Cipriani et al., 2018; Pilling et al., 2011). The widespread adoption of SSRIs is due in part to their advantageous side-effect profile with respect to earlier generation drug therapies, specifically tricyclics and MAO-inhibitors (De Wilde et al., 1993; López-Muñoz & Alamo, 2009; Tignol, 1993). Nevertheless, SSRIs have several disadvantageous properties such as being ineffective at combatting some of depression's symptoms such as motivational deficits and having high rates of treatment-resistance. These drugs are also associated with side-effects including sexual dysfunction and emotional blunting, whereby patients report deficits in the processing of both rewarding and aversive events (Cipriani et al., 2018; Gartlehner et al., 2011; Marazziti et al., 2019; Shelton & Tomarken, 2001; Warren, 2020).

The primary pharmacological action of SSRIs is inhibition of the 5-HT transporter (5-HTT; DeVane & Cl, 1999; J. Hyttel & Hyttel, 1994). This reduces the rate at which 5-HT is removed from the synapse, effectively increasing extracellular 5-HT in the DRN and forebrain regions (Bel & Artigas, 1992; Blier & Bouchard, 1994; Dankoski et al., 2016; Fritze et al., 2017; Haddjeri et al., 2004; Invernizzi et al., 1994; Rutter et al., 1994). It was originally thought that the therapeutic benefit of SSRI treatment resulted from globally increasing 5-HT levels, alleviating a deficiency in 5-HT transmission that formed the neurobiological basis of depressive symptoms (Blier & de Montigny, 1994; Coppen, 1967; Hirschfeld, 2000; López-Muñoz & Alamo, 2009; Ressler & Nemeroff, 2000). The association between 5-HT levels and depression is now controversial and at best likely to be an oversimplification. Thus, findings of 5-HT deficiencies in depression are inconsistent, and SSRIs take several weeks of administration to produce a full therapeutic effect and yet the drugs block the 5-HT transporter after a single administration (Cowen & Browning, 2015; Kendrick & Collinson, 2022; Moncrieff et al., 2022). Moreover, there is general agreement that

the therapeutic benefit of SSRIs likely arises from adaptive changes in 5-HT neuron function (see below), highlighting the need to fully understand the effects of repeated administration of these drugs on the dynamics of 5-HT neurons.

The effect of SSRIs on the dynamics of 5-HT neurons has been investigated through electrophysiological recordings of the DRN in rodents on SSRI treatment (Blier & de Montigny, 1994; Blier & El Mansari, 2013). This work suggests that the effect of SSRIs on 5-HT neuron firing depends on treatment duration. Thus, acute administration of SSRIs to treatment-naïve animals inhibits the firing of putative 5-HT neurons but when administered to animals having received a course of treatment over two to three weeks, this inhibitory effect is attenuated (Blier & de Montigny, 1983, 1994; Chaput, de Montigny, et al., 1986; Czachura & Rasmussen, 2000; Mansari et al., 2005). Similarly, the spontaneous firing rate of putative 5-HT neurons was reduced upon the onset of SSRI treatment but recovered with continued treatment (Chaput, de Montigny, et al., 1986; Czachura & Rasmussen, 2000). As a consequence of this adaptive change, it is widely assumed that during the course of SSRI treatment 5-HT signaling is gradually enhanced in forebrain regions. Indeed, following 14 days of SSRI treatment, responses of hippocampal neurons to electrical stimulation of the DRN were enhanced (Blier & Bouchard, 1994; Chaput et al., 1991) and concentrations of 5-HT have been reported to be elevated in multiple forebrain regions (Hjorth et al., 2000).

Desensitization of inhibitory 5-HT_{1A} autoreceptors is likely a contributor to these time-dependent effects of SSRIs on 5-HT neuron dynamics. Thus, single doses of SSRIs increase extracellular concentrations of 5-HT in the DRN (Bel & Artigas, 1992; Invernizzi et al., 1994) and inhibit the firing of DRN 5-HT neurons via a 5-HT_{1A}

autoreceptor mediated mechanism (Dong et al., 1999; Gartside et al., 1995; Mansari et al., 2005). Following continued SSRI treatment, the inhibitory effect of 5-HT_{1A} agonists on the firing of putative 5-HT neurons is attenuated (Blier & de Montigny, 1983; Chaput, de Montigny, et al., 1986; Czachura & Rasmussen, 2000; Mansari et al., 2005). Desensitization may be mediated by post-transcriptional modifications and internalization of 5-HT_{1A} autoreceptors (Descarries & Riad, 2012; Hensler, 2003; Raymond, 1991; Raymond et al., 1999). There is also evidence that chronic SSRI treatment desensitizes 5-HT_{1B/D} receptors which are expressed at 5-HT neuron nerve terminals and regulate quantities of released neurotransmitter (Moret & Briley, 2000; Sharp & Barnes, 2020).

The effect of SSRI treatment on 5-HT neuron dynamics may additionally result from their actions on other mechanisms of 5-HT neuron control (Hjorth, 2016). As outlined in previous sections, 5-HT neurons are under control from other neurons within the DRN and other brain regions. Many of these neuronal populations that control 5-HT activity are themselves sensitive to 5-HT (Sharp et al., 2007). For example, application of 5-HT_{2C} receptor agonists to the DRN inhibited 5-HT neurons likely via 5-HT-sensitive GABA neurons and activation of 5-HT₄ receptors in the PFC activated 5-HT neurons likely through a mechanism involving projections from the PFC to the DRN (Boothman et al., 2006; Lucas & Debonnel, 2002; Sharp et al., 2007). As SSRI treatment increases extracellular concentrations of 5-HT both in forebrain regions such as the PFC as well as the DRN (Bel & Artigas, 1992; Ceglia et al., 2004), 5-HT may modulate the activity of these 5-HT sensitive neuronal populations and indirectly effect the firing of 5-HT neurons.

Recent work indicates that there is diversity among 5-HT neurons with respect to both the expression of 5-HT_{1A} autoreceptors (Ren et al., 2019) and their connectivity to negative feedback pathways such as those arising from PFC neurons and local GABA neurons (Weissbourd et al., 2014). This could potentially result in subpopulations of 5-HT neurons being affected in different ways by SSRI treatment. However, to our knowledge, there are no existing electrophysiological studies that have reported on the diversity of responses among DRN 5-HT and non-5-HT neurons to SSRI treatment.

Although previous electrophysiological investigations have found that SSRIs affect the baseline activity of 5-HT neurons, it is not known how these drugs affect their responsivity to external inputs. In particular, one aspect of the activity of 5-HT neurons that may be particularly affected by SSRI treatment is the way in which they respond to aversive stimuli. Treatment with SSRIs is associated with deficits in aversive processing among both depressed patients and healthy controls (Marazziti et al., 2019; McCabe et al., 2010). Previous research in humans has linked such SSRI-associated deficits in emotional processing to altered fMRI signals in the amygdala evoked from aversive stimuli (McCabe et al., 2010). As outlined in previous sections, aversive stimuli also affect the firing of 5-HT neurons (Cohen et al., 2015; Hayashi et al., 2015; Schweimer & Ungless, 2010), but how this is influenced by SSRI treatment is unknown.

1.3 Large-scale neuronal recordings

In vivo electrophysiological studies of DRN 5-HT neurons have typically used electrodes designed to record extracellularly from a single neuron at a time such as glass micropipettes and tungsten wires. The next section introduces recent

developments in electrode technology that enable large numbers of neurons to be recorded simultaneously. Also discussed are the potential benefits and drawbacks of such large-scale recordings for the analysis of single DRN neurons and network dynamics.

1.3.1 Methods for large-scale neuronal recordings

There has been considerable progress in recent years in the fabrication of electrodes for extracellular electrophysiological recordings (Steinmetz et al., 2018). Indeed, due in large part to progress in electrophysiological methods, the largest number of simultaneously recorded neurons reported in indexed neuroscience studies grew exponentially between the 1960s and 2011, doubling approximately every 7.4 years (Stevenson & Kording, 2011).

The first electrodes used widely in vivo in mammals were metal microelectrodes made from indium and tungsten. These typically record from one neuron at a time. When placed in close proximity to a neuron, these electrodes can amplify and isolate its spikes relative to those arising from more distant neurons (Dowben et al., 1953; Hubel & Hubel, 1957; Steinmetz et al., 2018). Such fine wire electrodes, as well as others designed to record from single neurons such as glass micropipettes have been frequently used in electrophysiological studies of the DRN (Aghajanian et al., 1968; Allers & Sharp, 2003; Heym et al., 1982; Kirby et al., 2003; Schweimer & Ungless, 2010).

Tetrodes were the first widely used electrode technology for in vivo simultaneous recordings of multiple isolated neurons (Buzsáki, 2004). Tetrodes consist of one or more bundles of larger, lower impedance electrode wires packed close together. Spike

sorting algorithms designed for tetrode recordings use systematic differences in spike amplitudes across recording sites to partition spikes to putative single neurons (Buzsáki, 2004; Carlson & Carin, 2019; Steinmetz et al., 2018; Voigts et al., 2013).

One disadvantage of tetrodes is that in order to increase the number of neurons recorded more wire bundles must be included in the device (Steinmetz et al., 2018). A large number of independent wires can lead to relatively bulky designs which in turn render it difficult to target recording electrodes to small brain structures. Another disadvantageous property is the lack of rigidity of wire bundles. Anecdotal observations from the current laboratory show that tetrode wire bundles are prone to bend before reaching deep brain structures such as the DRN. Possibly due to these disadvantages, tetrodes have not been widely applied to in vivo electrophysiological studies of the DRN, with several notable exceptions (Cohen et al., 2015; Z. Liu et al., 2014; Ranade & Mainen, 2009; Waterhouse et al., 2004).

Silicon probe electrodes were developed to increase recording channel count while maintaining a small size and rigid construction. These electrodes are constructed using silicon microfabrication and miniaturization and can have many tens of recording sites on a single electrode shank (Steinmetz et al., 2018). Current state-of-the-art silicon probes can have hundreds of recording sites which is achieved by incorporating analog-to-digital conversion and signal multiplexing on the probe itself, thereby reducing the space requirement for wires that connect recording channels to downstream equipment (Jun et al., 2017; Steinmetz et al., 2018). Electrophysiological studies employing both ‘active’ and ‘passive’ silicon probes (i.e., those with and without on-probe multiplexing) have reported that these electrodes

have the capacity to record from tens to hundreds of neurons simultaneously (Steinmetz et al., 2018; Stevenson & Kording, 2011).

One disadvantage of active silicon probes when aiming to record from a specific brain region is the large spread of recording sites. Whereas the recording contacts of most passive probes are clustered at the probe tip, many active probes have recording sites that span relatively large distances. For example, the channels of Neuropixels, which are currently among the most commonly used active silicon probes, span almost 4 mm (Jun et al., 2017; Steinmetz et al., 2018), which is almost four times the depth of the mouse DRN (Michelsen et al., 2007). Due to the small dimensions of the mouse DRN, passive silicon probes may be better suited to large scale neuronal recordings in this brain region.

Spike sorting methods have been developed specifically for data generated using silicon probes (Carlson & Carin, 2019). One challenge for these algorithms has been distortions resulting from ‘colliding spikes’. Colliding spikes occur when two neurons fire at the same time creating a waveform shape that can corrupt the feature space of spike sorting algorithms (Carlson & Carin, 2019). Such artefacts are more likely to be detected by silicon probes due to their dense packing of recording sites and low background noise levels relative to tetrodes (Steinmetz et al., 2018). Recently developed spike sorting algorithms such as Kilosort2, mitigate this problem by modeling the raw voltage trace as a combination of spike trains of single neurons and background noise (Carlson & Carin, 2019; Pachitariu et al., 2016, 2021). In this way, when a neuron is isolated its spike train can be subtracted from the voltage time series, mitigating the effect of colliding spikes.

There has been a significant research effort to increase the computational efficiency of spike sorting algorithms. Success has been found by performing computations in parallel, and many commonly used spike sorting algorithms including Kilosort2 have been optimized for use on graphical processing units (GPUs) and multi-core processors (Carlson & Carin, 2019; Pachitariu et al., 2016, 2021). These optimizations greatly reduce the time required for spike sorting and analysis, making it more feasible to process the large datasets generated by silicon probe recordings.

1.3.2 Advantages of large-scale recordings for analysis of the properties of single neurons

Many analysis methods used in electrophysiological studies employing single neuron recording methods are aimed at characterizing the properties of single neurons independently, for example their electrophysiological properties or tuning to a behavioral variable. However, the low neuronal yield of these approaches has often led researchers to focus recordings to neuronal subpopulations chosen *a priori*. Thus, many electrophysiological studies of the DRN have focused recordings to putative 5-HT neurons (i.e., slow, regular firing neurons with wide spike waveforms) and not reported on the properties of non-5-HT neurons [e.g., (Chaput, de Montigny, et al., 1986; Chaput et al., 1991; Mansari et al., 2005; Wilkinson & Jacobs, 1988)]. Another disadvantage of single neuron recording techniques is that they often generate datasets that lack the power to understand the diversity of properties of neurons within a population. In the case of the DRN, the vast majority electrophysiological studies to date report the properties of a ‘typical’ neuron rather than estimating the proportion of neurons within the population that respond in a particular way [e.g., (Schweimer et al., 2011; Schweimer & Ungless, 2010; Waterhouse et al., 2004)]. Large scale recordings may be able to overcome these disadvantages.

First, the large neuronal yield of the recordings increases dataset size and thus statistical power for analysis of functional diversity (Urai et al., 2021). For example, large scale recordings were recently applied to quantify the diversity of neuronal responses to visual stimuli in mouse visual cortex (de Vries et al., 2020; Siegle et al., 2021). In one study, putative neuron subtypes were identified by clustering neurons according to their response profiles to a set of visual stimuli and associations were identified between the density of putative neuron subtypes and localization within the cortical area (de Vries et al., 2020). A similar approach could be applied to the DRN for example to investigate the diversity of neuronal responses to aversive stimuli or SSRI treatment.

Second, because large-scale recording methods such as silicon probes sample large numbers of neurons, recordings can be obtained in an unbiased way rather than from hand-picked neuronal subpopulations (Urai et al., 2021). For example, one recent study used high channel count `Neuropixel` silicon probes to monitor the dynamics of ~30000 neurons located in multiple brain regions in mice performing a visually guided discrimination task (Steinmetz et al., 2019). Behavioral variables were identified that modulated activity in most neuronal populations in the same way, as well as others for which specific neuronal populations were specialized (Steinmetz et al., 2019). A similar approach could be applied to the DRN to investigate whether subpopulations of 5-HT or non-5-HT neurons are specialized for specific brain functions, for example aversive processing.

1.3.3 Analysis of neuronal network dynamics

Neuronal interactions, the relationships between the activity of neurons within a neuronal population, have long been hypothesized to be an important aspect of

neuronal dynamics (Yuste, 2015). However, they have been difficult to measure experimentally using traditional single neuron electrode technology – in order to quantify neuronal interactions, neurons must be recorded simultaneously (Cohen & Kohn, 2011). The widespread adoption of large-scale recording techniques such as silicon probes in recent years has coincided with a large research effort to identify and ascertain the functional significance of neuronal interactions. It is beyond the scope of this text to thoroughly review this literature. Instead, this section introduces two methodological approaches that have been commonly used in these studies that may be especially applicable to electrophysiological investigations of the DRN.

One approach that has been used in studies of neuronal interactions involves the analysis of pairwise neuronal correlations. Pairwise neuronal correlations can be quantified using statistical metrics that measure the similarity between activity time series of pairs of simultaneously recorded neurons. Pearson correlation (i.e., spike count correlation) has been one of the most commonly used metrics. Others include non-linear measures such as mutual information (Bettencourt et al., 2007; Singh & Lesica, 2010), time-series based measures such as cross correlation (Kohn & Smith, 2005; Totah et al., 2018) and transfer entropy (Nigam et al., 2016; Thivierge, 2014), as well as partial relationships i.e., modeling the relationship between the activity of two neurons having adjusted for other influences on neuronal dynamics such as common coupling to other neurons or external variables (Gerhard et al., 2011; Pillow et al., 2008). These statistical measures can be adapted to measure neuronal correlations occurring on different temporal scales (e.g., fast timescale synchronization of spike timings or slow timescale co-fluctuation of spike rates; Cohen & Kohn, 2011).

The neurobiological mechanisms that give rise to pairwise neuronal correlations have been investigated by both theoretical and experimental studies. The findings of this work indicates that neuronal correlations can reflect both (1) neurons having similar patterns of afferent input and (2) neurons being connected with each other directly (Cohen & Kohn, 2011; English et al., 2017; Ostojic et al., 2009). There is some evidence that slow and fast timescale correlations may result from different neurobiological mechanisms with fast timescale interactions being more likely to arise from direct connectivity (English et al., 2017; Smith & Kohn, 2008).

There have been several applications of analyses of pairwise neuronal correlations that may be relevant for electrophysiological investigations of the DRN. First, studies have quantified pairwise neuronal correlations across a range of timescales in order to investigate the temporal component of neuronal activity most related to neighboring neurons (Bair et al., 2001; Diba et al., 2014; Kodama et al., 2018; Okun et al., 2019; Smith et al., 2013). The findings of this work indicates that the timescale of neuronal interactions may differ by neuronal population and brain region. For example, fast timescale interactions have been identified between putative interneurons in the hippocampus with interacting neurons firing at millisecond latencies from each other (Diba et al., 2014). Conversely, slow timescale correlations of spike rate have been reported to be prominent in primary somatosensory cortex with fast timescale correlations being sparse in this region (Kodama et al., 2018). Additionally, there is evidence that neuronal correlations may differ across timescales: one recent electrophysiological study reported that cortical neurons that were most correlated with neighboring neurons at one timescale were not necessarily the same as those occurring on another (Okun et al., 2019). As mentioned in previous sections, 5-HT neurons can signal on multiple timescales (Cohen et al., 2015; Hayashi

et al., 2015), and are thought to be regulated by neighboring neurons within the DRN (Sharp et al., 2007). It is not known whether different temporal components of the 5-HT signal are particularly sensitive to local network dynamics.

Second, analyses of pairwise correlations have been employed to investigate the relationships occurring between neuron types in heterogeneous neuronal populations. Several studies have used this approach to investigate the relationships between the dynamics of excitatory and inhibitory neurons (Adler et al., 2013; Hofer et al., 2011; Karnani et al., 2016). For example, one study demonstrated that the firing of striatal medium spiny projection neurons was positively correlated with one class of local interneuron and uncorrelated with another, consistent with these interneurons regulating stratal output in different ways (Adler et al., 2013). Other studies have investigated the diversity of neuronal correlations occurring within neuron types with heterogeneous functional properties. For example, it has commonly been reported that principal neurons in primary visual cortex have higher pairwise neuronal correlations with neighboring neurons with similar stimulus response profiles (Cohen & Kohn, 2011; Hofer et al., 2011; Kohn & Smith, 2005). Applied to the DRN, analyses of pairwise neuronal correlations may thus enable electrophysiological studies to investigate (1) the control of 5-HT neuron dynamics by local GABA neurons or (2) the interactions occurring between 5-HT neurons with diverse anatomical and functional properties.

Another methodological approach that may benefit electrophysiological studies of the DRN is the application of analysis methods from network science, wherein neuronal populations are represented as graphs comprising nodes and edges corresponding to neurons and the interactions between them, respectively (Bassett & Sporns, 2017;

Humphries, 2018). Community detection algorithms are one class of network analysis method that have been frequently applied to investigations of neuronal network dynamics. They are designed to group nodes into clusters with high interactivity and have been applied to data from large scale recordings to identify neuronal ensembles (Humphries, 2011, 2018; Newman, 2010). As mentioned in previous sections, neuronal ensembles have been identified in multiple brain regions including the LC, amygdala, and primary somatosensory cortex (Fustiñana et al., 2021; Humphries, 2011; Kodama et al., 2018; Totah et al., 2018).

Studies have investigated the properties of neuronal ensembles such as their neuron type composition and relationship to behavioral outputs. The findings of this work indicates that neuronal ensembles in heterogeneous neuronal populations may be preferentially comprised of specific neuron types with similar effects on neural processing and behavior. For example, recordings in primary visual cortex reported that VIP and SOM interneurons formed co-active neuronal ensembles that predominantly contained other interneurons (Karnani et al., 2016). Another study reported that putative noradrenergic neurons in the LC were more likely to form neuronal ensembles with neurons with similar waveform characteristics and anatomical connectivity (Totah et al., 2018). Further analysis of LC ensembles indicated that periods of heightened activity in different ensembles were associated with different patterns of cortical activity, as indexed by LFP (Shahryar Noei et al., 2022). Consistent with ensembles representing functional modules, one study reported that basolateral amygdalar neurons formed two anticorrelated neuronal ensembles each consisting of neurons with similar tuning to exploratory and social behavioral outputs (Fustiñana et al., 2021). As mentioned in previous sections, 5-HT neurons located in the DRN have diverse molecular, anatomical, and functional

properties. Network analysis approaches such as the analysis of neuronal ensembles may be a promising avenue to ascertain how this variation is expressed in the dynamics of these neurons.

1.3.4 Analysis of population coding

Population coding is a neural coding framework that posits that neuronal populations encode cognitive and behavioral variables in their joint activity (Saxena & Cunningham, 2019; Yuste, 2015). It is in contrast to single neuron coding frameworks which instead posit that such variables are encoded independently by individual neurons (Yuste, 2015). Though theories of population coding have existed for many years (Hebb, 1949), experimental investigations at this time were limited by the inability of available recording methods to monitor the activity of groups of neurons simultaneously (Yuste, 2015). The widespread adoption of large-scale recording methods such as silicon probes in recent years has mitigated this limitation and there has been substantial research investigating the prevalence and mechanisms of population coding (Saxena & Cunningham, 2019; Urai et al., 2021). Although an exhaustive review of this work is beyond the scope of this text, this section introduces some of its major findings and outlines methodological approaches used that may be applicable to electrophysiological investigations of the DRN.

Electrophysiological studies have investigated whether neuronal populations use a population coding scheme by comparing the performance of stimulus decoding models trained on groups of neurons to those trained on single neurons (i.e., population and single neuron decoders, respectively; Nicolelis & Lebedev, 2009). Improved performance of population decoders over single neuron decoders has been interpreted as evidence for the neuronal population using a population coding

scheme. Using similar methodological approaches to this, evidence for population coding has been reported for sensory (Gutierrez et al., 2006; Hung et al., 2005; Katz et al., 2002; Runyan et al., 2017; Simon et al., 2006), motor (Georgopoulos et al., 1986; Nicolelis & Lebedev, 2009; Shenoy et al., 2003), and cognitive (Kaufman et al., 2022; Rigotti et al., 2013; Runyan et al., 2017; Saxena & Cunningham, 2019) variables. For example, one study performed simultaneous neuronal recordings in rat motor cortex during seven days of training on a behavioral task (Laubach et al., 2000). On each training day, the capacity of population and single neuron decoders to predict trial outcomes was assessed. The performance of population decoders improved as rats learned the behavioral task while that of single neuron decoders was not above chance levels at neither the beginning nor end of training. This was interpreted as evidence for motor cortical neurons encoding the behavior using a population coding scheme.

There has been a large research effort to identify the potential brain function served by population coding schemes and how they relate to the well-studied properties of single neurons. Population coding has been proposed to be a mechanism through which the activity of neurons that are tuned broadly to behavioral variables can be combined to generate fine outputs. For example, one study performed electrophysiological recordings in monkey motor cortex during a behavioral task involving precise arm movements (Georgopoulos et al., 1986). Such precise behavioral outputs could not be decoded from the activity of single neurons which were generally tuned broadly to a range of arm movements. However, accurate predictions could be made from population decoders that performed a weighted sum over the tuning curves of single neurons.

In sensory cortex, population coding has been proposed to mitigate the trial-to-trial variability of neuronal firing to stimuli. For example, one recent study employed two-photon calcium imaging to record from visual cortical neurons in mice during a visually guided discrimination task (Stringer et al., 2021). Although single neurons were observed that were tuned to specific stimuli, their evoked activity varied over trials, despite accurate detection of the stimulus, as indexed by the mouse's behavioral performance. Linear decoders could accurately predict visual stimuli from population activity, achieving performance that had less trial-by-trial variance, matching and even exceeding behavior.

Another function that has been ascribed to population coding has been the expansion of the representational capacity of neuronal populations (Fusi et al., 2016; Kaufman et al., 2022). In many brain regions, the firing of neurons is believed to represent information about multiple cognitive and behavioral variables. For example, and as mentioned previously, the firing of DRN 5-HT neurons has been linked to behavioral states such as arousal, rates of rewards in the current environment, and specific aversive stimuli (Cohen et al., 2015; Hayashi et al., 2015; Trulsson & Jacobs, 1979). Under the assumption that neuronal activity is read out using linear decoders, the number of possible variables that can be encoded by a neuronal population can increase when neurons use a population- with respect to a single neuron- coding scheme (Fusi et al., 2016). Specifically, representational expansion occurs when neurons exhibit non-linear mixed selectivity whereby neurons are selectively activated by combinations of different cognitive and behavioral variables that cannot be predicted by their responses to individual variables (e.g., a neuron being activated by two discrete behavioral variables independently whose response when both variables are present is not a summation of its response to either alone).

Neurons with non-linear mixed selectivity have been identified in several brain regions including the PFC, entorhinal cortex and hippocampus (Boyle et al., 2022; Hardcastle et al., 2017; Rigotti et al., 2013). Evidence for mixed selectivity neurons contributing to a population code was provided by one study that monitored groups of PFC neurons in monkeys performing a sequence memory task (Rigotti et al., 2013). Many neurons exhibited heterogeneous non-linear mixed selectivity, and it was not possible for single neuron decoders to predict specific behavioral outputs. However, due to the diversity of combinations of task variables to which mixed selective neurons were tuned, population decoders could accurately decode behavioral performance.

Although the effects of sensory stimuli and behavioral outputs on the firing of DRN 5-HT neurons have been studied extensively, there have, to our knowledge, not yet been any electrophysiological studies that have investigated whether these neurons encode these variables using population coding mechanisms. This is likely due to the small number of studies that have measured the simultaneous dynamics of groups of DRN neurons.

1.4 Aims of this thesis

Experiments in this thesis utilized for the first time, silicon probes to generate large-scale recordings of DRN neurons directed towards i) quantifying the diversity of neuronal firing properties in this region, ii) assessing emergent population dynamics amongst DRN neurons such as neuronal interactions and population coding, and iii) determining the effect on these parameters of specific aversive stimuli and pharmacological manipulations.

In Chapter 2, the potential for silicon probes to generate large-scale recordings of DRN neurons was investigated. Recordings were made in mice under urethane anesthesia, and the targeting of silicon probes to the DRN was assessed through histological analysis of electrode tracts. The firing properties of individual DRN neurons were identified through spike-sorting and subject to clustering analysis, and the dataset generated was compared with a second dataset of DRN neurons obtained with glass micropipettes. Experiments also investigated the response of putative 5-HT neurons and other neuron types to pharmacological manipulation, specifically acute administration of the SSRI citalopram.

In Chapter 3, a subset of the dataset of Chapter 2 was further analyzed to investigate the effect on DRN 5-HT neurons of an aversive foot-shock stimulus. The diversity of responses to foot-shocks among both putative 5-HT and non-5-HT neurons was investigated. The likelihood that DRN population responses were operating according to a population code was assessed using predictive models.

Chapter 4 also further analyzed the dataset of Chapter 2. Analyses leveraged the simultaneous nature of silicon probe recordings to quantify DRN network dynamics. Interactions between putative 5-HT neurons and non-5-HT neurons were investigated by analyzing distributions of pairwise spike count correlations. Additionally, analyses were conducted to determine whether neuronal activity within the DRN had a modular structure and contained neuronal ensembles.

Finally, in Chapter 5 silicon probe recordings were utilized to investigate the effect of chronic SSRI treatment on the DRN neuronal network. A second dataset was collected of electrophysiological measurements in urethane anesthetized mice having been treated with either citalopram or saline for two weeks prior to the recording. Large

scale recordings investigated whether citalopram treatment was associated with differential effects on putative 5-HT neurons and impacted the activity of putative non-5-HT neurons in the DRN network. Moreover, analyses were conducted to determine whether citalopram treatment affected DRN neuronal interactions and sensitivity to an aversive foot shock stimulus.

Chapter 2: Silicon probe recordings of dorsal raphe nucleus neurons

2.1 Introduction

2.1.1 Electrophysiological studies of the dorsal raphe nucleus 5-HT system

The DRN contains the largest number of 5-HT neurons in the brain (Michelsen et al., 2007). *In vivo* electrophysiological recordings, which enable the activity of isolated neurons to be monitored, have been one of the primary approaches used to investigate the properties of DRN 5-HT neurons (Jacobs & Fornal, 1999). The findings of such electrophysiological studies of the DRN have informed theories of 5-HT-neuron function. For example, electrophysiological recordings of DRN 5-HT neurons during presentations of aversive and rewarding stimuli revealed complex tuning of 5-HT neurons over multiple timescales (Cohen et al., 2015), a result which led to the re-evaluation of theories of the role of 5-HT neurons in emotional processing (Dayan et al., 2015).

Most previous *in vivo* electrophysiological studies of the DRN have employed single unit extracellular recordings [e.g., (Jacobs & Azmitia, 1992; Sakai & Crochet, 2001; Schweimer & Ungless, 2010; Steinmetz et al., 2018)]. Single unit electrophysiological recordings are designed to record from a single neuron at a time, utilizing electrodes such as glass micropipettes and tungsten wires. As outlined in Chapter 1, recording from only one neuron at a time can impose several constraints and limitations. For

example, they preclude the measurement of population dynamics and often generate datasets of insufficient size to characterize the heterogeneity of physiological properties within neuronal populations (Urai et al., 2021).

2.1.2 Large-scale electrophysiological recordings

Multi-channel electrodes enable *in vivo* electrophysiological recordings of multiple neurons simultaneously. However, as outlined in Chapter 1, until recently, the size and rigidity of the most common multi-channel electrodes hindered their application to small subcortical nuclei, such as the DRN. Moreover, the process of sorting spikes recorded with these electrodes to isolated single neurons (i.e., spike sorting) was a challenge.

There has been substantial progress in recent years in the development of multi-channel electrodes and the algorithms for spike sorting signals recorded with them. (Buzsáki, 2004; Carlson & Carin, 2019; Pachitariu et al., 2021; Steinmetz et al., 2019). In particular, silicon probes are a novel electrode design containing multiple recording channels (typically 16-64 channels) in close proximity on a single electrode shank and have been demonstrated to enable simultaneous multiple neuron recordings (Steinmetz et al., 2018). This design is advantageous for small, deep-lying brain regions such as the DRN which are difficult to target using bulkier multiunit electrode designs such as tetrodes and recently developed ‘active’ silicon probes such as ‘Neuropixels’ (Jun et al., 2017; Michelsen et al., 2007).

Recent studies have demonstrated the capability of silicon probes to record electrical activity in small brain structures in rodents and, following spike sorting of the electrode signals, generate simultaneous multi-neuron recordings (Berényi et al.,

2014; Bragin et al., 2000; Sales et al., 2021; Steinmetz et al., 2018; Ulyanova et al., 2019).

2.1.3 Identifying 5-HT and GABA neurons

The DRN is a highly heterogeneous brain region. 5-HT neurons comprise 35-50% of the neuron population, and these neurons are embedded amongst multiple other neuron types including glutamate, dopamine, GABA, and peptide neurons (Baker et al., 1990; Belin et al., 1979; Descarries et al., 1982; Kaneko et al., 1990; Lu et al., 2006; Michelsen et al., 2007; Wiklund et al., 1981). Thus, in order to investigate the properties of 5-HT and GABA neurons through *in vivo* electrophysiological recordings of DRN neurons, they must be identified amongst other DRN neuron types.

Whilst electrophysiological measurements do not enable direct measurement of neurochemical identity (Buzsáki, 2004), there has been a large research effort to uncover electrophysiological properties of DRN neurons that are predictive of 5-HT and GABA neurochemical identity. Electrophysiological studies of the DRN have commonly reported neurons with three electrophysiological profiles: (1) slow and regular firing neurons with broad spike waveforms (2) slow and irregular firing neurons without broad spike waveforms, and (3) fast-firing neurons with narrow spike waveforms (Aghajanian et al., 1968; Allers & Sharp, 2003; Jacobs & Azmitia, 1992; Nakahama et al., 1981; Sakai & Crochet, 2001; Schweimer et al., 2011; Schweimer & Ungless, 2010). There is evidence that some of these electrophysiological profiles are predictive neurons being 5-HT and GABA neurons. This evidence has come from studies which combined electrophysiological recordings with juxtacellular labelling, and subsequent immunocytochemical analysis of labelled

cells (Pinault, 1996). Using this approach several studies have reported that most slow regular firing DRN neurons with broad waveforms were immunopositive for markers of 5-HT neurons whereas most fast-firing DRN neurons with narrow waveforms were immunopositive for GABA markers (Allers & Sharp, 2003; Hajós et al., 2007; Schweimer et al., 2011; Schweimer & Ungless, 2010).

The pharmacological properties of DRN neurons also help predict their neurochemical identity. In particular, 5-HT neurons are thought to be differentially inhibited by 5-HT_{1A} receptor agonists and drugs that indirectly increase concentrations of 5-HT in the DRN such as SSRIs. Evidence for this idea comes from histological and transcriptomic studies that have reported that most DRN 5-HT neurons express inhibitory 5-HT_{1A} autoreceptors and most non-5HT neurons do not (Huang et al., 2019; Sotelo et al., 1990), as well as from electrophysiological studies that have reported that slow regular firing DRN neurons with broad spike waveforms are inhibited by administration of 5-HT_{1A} receptor agonists and SSRIs (Jacobs & Azmitia, 1992; Scuvée-Moreau & Dresse, 1979; Vandermaelen & Aghajanian, 1983). Furthermore, *in vitro* electrophysiological studies indicate that most non-5-HT DRN neurons lack expression of 5-HT_{1A} receptors and are generally not inhibited by 5-HT_{1A} agonists (Kirby et al., 2003).

Several studies have used a combination of electrophysiological and pharmacological properties to estimate the identity of 5-HT neurons in electrophysiological studies of the DRN. For example, one behavioral study classified a sub-population of DRN neurons as being 5-HT neurons on the basis of their slow regular firing pattern and broad spike waveforms and being sensitive to inhibition via a 5-HT_{1A} receptor agonist (Waterhouse et al., 2004).

It is important to verify that neurons recorded with silicon probes have equivalent electrophysiological and pharmacological properties to those identified in electrophysiological studies using traditional electrode designs. This is because certain electrophysiological properties such as waveform shape may be sensitive to changes in the properties of electrodes such as the impedance of recording channels and the distance between the electrode and recorded neuron (Fiáth et al., 2021; Gold et al., 2006).

2.1.4 Chapter aims

Through electrophysiological recordings in urethane anesthetized mice, the aims of this study were (1) to determine whether silicon probe electrodes could be used to generate large-scale recordings of DRN neurons, and (2) to characterize the electrophysiological properties of silicon probe recorded neurons with a view to potentially estimating the identity of 5-HT and GABA neurons from these recordings.

The capacity to target silicon probes to the DRN and generate large scale recordings was assessed via histological analysis of electrode tracks and quantification of neuronal yield following spike sorting of electrode signals.

The electrophysiological properties of silicon probe recorded neurons were established for comparison with both previous descriptions of identified 5-HT and GABA DRN neurons in the literature as well as a second dataset of single unit recordings collected using glass micropipette electrodes.

To test whether putative 5-HT neurons had pharmacological properties consistent with them being a majority 5-HT population, experiments tested for inhibition by an acute challenge with the SSRI citalopram.

2.2 Methods

2.2.1 Animals

Male adult wild type mice (C57BL6/J) were used in all experiments. Animals were group housed, placed on a 12-hour light/dark cycle and provided food and water *ad libitum*. All procedures were performed in accordance with the Animal (Scientific Procedures) Act 1986 (ASPA, UK) and within the guidelines of the University of Oxford.

2.2.2 Datasets

Two datasets were collected. The first dataset was multi-neuron recordings of DRN neurons made using silicon probe electrodes in urethane anesthetized mice. The second dataset was single neuron recording of DRN neurons made using glass micro-pipette electrodes in urethane anesthetized mice.

2.2.3 Generation of silicon probe recordings

2.2.3.1 Hardware

The hardware used to record from DRN neurons using silicon probes is shown in Methods Figure 2.1. Recordings were obtained using double shank, 32-channel silicon probes (Cambridge Neurotech ‘P Series’) attached to an Intan RHD2000 series head-stage preamplifier that converted the signals from analog to digital and multiplexed them. The Intan head-stage was connected by an Intan SPI cable to an Open Ephys recording box that controlled signal acquisition (Siegle et al., 2017).

2.2.3.2 *Surgery and electrode implantation*

All recordings were obtained from urethane anesthetized mice. Mice were initially anaesthetized with isoflurane and then maintained with urethane (1.7 mg/kg, i.p.). The scalp was shaved, and a local anesthetic was applied (bupivacaine Marcaine, Accord Healthcare UK, s.c.) prior to placing the animal in a stereotaxic frame (Kopf Instruments). To prevent damage to the eyes caused by drying, eye ointment was applied locally (Lacrilube, Allercan). Body temperature was controlled via a homeothermic heating blanket connected to a rectal thermometer. The scalp was exposed and washed with sterile saline prior to a craniotomy being performed over the DRN. The silicon probe was mounted on the stereotaxic frame (NeuroNexus A32-Omnetrics adaptor) at a 10-degree angle and gently lowered into the DRN (stereotaxic coordinates versus bregma; -4.5 mm AP, -0.5 mm ML, 2.5 mm DV).

2.2.3.3 *Electrode localization and histology*

Prior to implantation probes were coated in a lipophilic fluorescent dye (DII) to allow *post-hoc* localization of electrode tracts by a histological analysis. At the end of each recording session mice were transcardially perfused with cold phosphate buffered saline (PBS) followed by 4% PFA in PBS (total volume of each solution was approximately 10mL). Brains were extracted, stored overnight in 4% PFA and the next day transferred to sucrose solution (30% in PBS) for cryoprotection. The following day brains were transferred to PBS solution and stored until sectioning.

For tissue sectioning, 30 μ m coronal sections were cut using a cryostat from the region around the DRN (approximately -3.88 to -5mm AP) and stored floating in PBS on 24-well plates. For histological analyses, sections were washed in PBS (3x washes, 5 min each) and mounted on un-coated slides and cover-slipped using a mounting medium

(Vector Laboratories, Peterborough UK) prior to imaging using an epi-fluorescent microscope.

Decisions to include or exclude recording sessions were made considering both histological evidence for electrode placement and physiological evidence for DRN-specific electrophysiological profiles. Recording sessions were included if DII-derived fluorescence was identified in the DRN. Where such evidence was unavailable, sessions were excluded if no neurons with a slow and regular spontaneous firing pattern were recorded.

2.2.3.4 Data processing

Silicon probes generated 32-parallel voltage time-series at a 30kHz sampling rate. The raw data were spike sorted before being analyzed.

Spike sorting was performed in a two-step process. In the first step, Kilosort2 was used to automatically detect spikes and assign them to putative isolated neurons (Pachitariu et al., 2016, 2021). Kilosort2 was used as it adapts traditional spike-sorting algorithms to leverage the advantages and mitigate the disadvantages of data generated with silicon probes. Advantages of Kilosort2 over traditional waveform clustering algorithms include the use of a template-matching approach to recover overlapping spikes, the use of neighboring channels to amplify local signals, and a procedure to mitigate the effects of electrode drift (Carlson & Carin, 2019; Pachitariu et al., 2016, 2021). In the second step, the quality of each putative single neuron was manually inspected using the Phy software package. Metrics used to determine single-unit status during manual curation were auto-correlation values at short time lags, waveform shape, and spike amplitude to noise ratio. These metrics have been

proposed to correlate with spike-sorting quality (Carlson & Carin, 2019; Hill et al., 2011).

Spike-sorted spike trains were stored in a MySQL database for later analysis. For many analyses, spike trains were converted to ‘spike vectors’, regularly sampled time-series with one entry for each neuron. The value at each entry was the number of spikes produced by that neuron during a time bin (Carrillo-Reid et al., 2017).

2.2.4 Generation of glass-micropipette single unit recordings

2.2.4.1 Hardware

The hardware used to record from DRN neurons using glass micropipettes is shown in Methods Table 1. Neuronal signals were recorded with custom-built glass micropipettes (8-60 M Ω) filled with 1.5% neurobiotin in 0.5M NaCl. Analog signals were streamed from the electrode to an amplifier. To remove noise, signals were subject to spectral filtering between 300-5000 Hz and removal of electrical mains noise. Finally, signals were converted from analog to digital and streamed to a personal computer for storage. Data acquisition was controlled by the computer via the Spike2 software package (Cambridge Electronic Design, Cambridge UK). All signals were sampled at 10 kHz.

2.2.4.2 Surgery and implantation

All single unit recordings were made in urethane-anaesthetized mice using a similar surgical procedure to that outlined above. After performing the craniotomy, glass micropipettes were mounted on a microdrive fixed to the stereotaxic frame (zero-degree angle) to allow a controlled electrode descent to the DRN (stereotaxic coordinates relative to bregma; -4.5 mm AP, 0 mm ML, 2.5 mm DV).

2.2.4.3 Recording protocol

All sessions ($n = 26$) had an initial 30-min block during which spontaneous activity was recorded. A subset of sessions ($n = 10$) additionally had two further 20-min blocks during which neuronal activity was monitored following the administration of pharmacological agents (detailed below).

2.2.4.4 Electrode localization and histology

Recording electrodes were filled with neurobiotin which was expelled by high current at the end of the experiment to allow localization of the electrode. Recordings were included if electrode descents were revealed to be in the DRN. Where histology was unavailable, the electrophysiological characteristics of recorded neurons were used as exclusion criteria. Electrode descents on which no slow and regular spiking neurons were recorded were excluded.

For detection of neurobiotin, brains were first fixed and sectioned as outlined above. To visualize neurobiotin sections were then incubated for 2 h with streptavidin conjugated to Alexa-488. Sections were then washed (3x washes, 5 min each) and mounted on un-coated slides and cover slipped using a mounting medium. (Vector Laboratories, Peterborough UK) prior to imaging using an epi-fluorescent microscope.

2.2.4.5 Data processing

Data were stored as single channel 10kHz voltage time-series. Spikes were detected using a threshold-crossing procedure and sorted using a waveform-clustering algorithm implemented in the Spike2 software package. Spike trains were exported from Spike2 and stored in a MySQL database for further analysis.

2.2.5 Analysis of electrophysiological characteristics

2.2.5.1 Design

The electrophysiological properties of silicon probe recorded neurons were analyzed in order to determine whether they were consistent with those in the glass micropipette dataset. The degree of consistency was assessed by first clustering neurons into groups sharing electrophysiological properties (referred to as ‘neuron types’ – see below) and then (1) comparing the relative frequency of neuron types across datasets, and (2) comparing the electrophysiological properties of neurons of each neuron type in the two datasets.

2.2.5.2 Dataset

The electrophysiological properties of neurons were investigated during spontaneous activity. For silicon probe recordings, spontaneous activity was recorded for 30 mins. For glass pipette recordings, each single neuron was recorded for a minimum of 4 mins.

2.2.5.3 Calculation of electrophysiological properties

Spike rate was calculated as the number of spikes per second. Spike regularity was parameterized as the CV2 of inter-spike intervals. The CV2 was used as it has been demonstrated to perform better on neural recordings over long time periods than the coefficient of variation (Holt et al., 1996). Average waveforms were generated for each neuron by computing the mean voltage on the channel at which it had maximum amplitude at all offsets ± 5 ms from spike initiation over 1000 spikes. Waveform width was then computed from average waveforms as the time difference between spike initialization and repolarization. Waveform inflection points were obtained using the

peak-finding algorithm implemented in the Python package ‘SciPy’ (Virtanen et al., 2020).

$$Cv2 := \frac{1}{N} \sum_{i=1}^{N-1} \frac{2|I_{i+1} - I_i|}{|I_{i+1} + I_i|}$$

Equation 2.1: Cv2

Metric used to calculate spike regularity. $\mathbf{I}$ is a vector of time-ordered inter-spike intervals from one neuron.

2.2.5.4 *Neuron clustering using electrophysiological characteristics*

Neurons in the silicon probe dataset were clustered on the basis of their electrophysiological characteristics using the k-means algorithm ($k = 3$). Values were standardized prior to clustering. Neuron clusters in the glass-micropipette dataset were classified into the neuron types defined by the silicon probe dataset. Neurons were first transformed to be on the same scale and then were classified by applying the decision boundary of the fit k-means model. Clustering was implemented using the ‘scikit-learn’ python package (Pedregosa et al., 2012). Clusters resulting from the above process are referred to as ‘neuron types’.

2.2.5.5 *Comparison between datasets*

Differences in the proportion of neuron types were assessed using a chi-square contingency test. For each metric, between dataset differences were assessed using linear models with neuron type and metric as dependent variables and metric value as independent variables. The presence of main effects was determined using F-tests.

Tukey t-tests were used as *post-hoc* tests where applicable. Statistical analyses were performed using the ‘SciPy’ python package, ‘stats’ R package and ‘emmeans’ R packages (Lenth, 2022; Virtanen et al., 2020).

2.2.5.6 Volatility of electrophysiological characteristics

The degree to which the electrophysiological characteristics of neurons in the silicon probe dataset varied over time was quantified using a volatility metric. Volatility was computed by first breaking down the 30-min spontaneous activity block into 15 non-overlapping 2-min bins, calculating the metric of interest (e.g., firing rate) in each bin, and then computing the coefficient of variation over bins. Neurons with high volatility for some metrics (e.g., firing rate) had variable values in that metric over time.

2.2.6 Determining the effects of acute citalopram challenge on DRN neuron activity

2.2.6.1 Design

Experiments were designed to determine whether silicon probe recorded DRN neurons were inhibited by acute systemic administration of the SSRI citalopram via a 5-HT_{1A}-dependent mechanism. It was predicted that slow regular firing neurons (i.e., putative 5-HT neurons) would be more sensitive to citalopram than slow irregular and fast-firing neurons (i.e., putative non-5-HT neurons).

Mice were randomly assigned to one of two groups: saline (n = 3 mice) or 10 mg/kg i.p. citalopram (n = 7 mice). Mice were first administered either saline or citalopram, and then 40 min later all animals received 1 mg/kg i.p. WAY100365, the selective 5-HT_{1A} receptor antagonist.

2.2.6.2 *Drugs*

Both citalopram hydrobromide (Abcam) and WAY100365 (Abcam) were dissolved in sterile saline to a concentration of 1 mg/ml. Drugs were stored in the solid form and made up on the day of the experiment.

2.2.6.3 *Statistical analysis of drug effects*

To determine drug effects, firing activity during a 10 min pre-drug block of spontaneous activity was compared to that occurring 20 min following drug administration. Effects were analyzed using linear mixed-effects models, where the dependent variable was the spike count of a neuron during a time bin (30 sec duration). For both models, neuron ID was the only random effect.

The fixed effects of the model for the first drug administered (i.e., saline or citalopram) were block (pre- versus post-drug administration), group (saline or citalopram) and neuron type and all corresponding interaction terms.

The fixed effects of the model for the second drug administered (i.e., WAY 100365) were block (pre- versus post-WAY100365 administration), group (prior administration with saline or citalopram) and neuron type and all corresponding interaction terms.

Models were fit using the ‘lme4’ R package, tests for fixed effects were computed using the ‘LmerTest’ package, and contrasts for *post-hoc* tests were computed using the ‘emmeans’ package (Bates et al., 2015; Kuznetsova et al., 2017; Lenth, 2022).

2.2.7 Software and statistics

Data processing, transformations, and statistical analysis were performed using custom-written software in R, Python, MATLAB, and SQL. All statistical analysis used an alpha level of 0.05 and multiple comparisons were accounted for where appropriate using Tukey and Benjamini-Hochberg adjustments.

2.3 Results

2.3.1 Silicon probe recordings of DRN neurons

Silicon probe recordings were performed in urethane-anaesthetized mice ($n = 26$). Successful localization of silicon probes was established by histological analysis which revealed fluorescent tracts extending from the brain surface and terminating in the DRN (Figure 2.1- A).

A two-step procedure was used to sort silicon probe recorded spikes into putative single units (see Methods; putative single units are hereafter referred to as single neurons). Using this approach, it was possible to isolate multiple single neurons simultaneously: mean neurons per session: 25 ± 2.7 (Figure 2.1 – B). In these experiments a total of 566 carefully sorted and curated DRN neurons were recorded.

2.3.2 Electrophysiological properties of silicon-probe recorded DRN neurons

To reveal the most common electrophysiological profiles of silicon probe recorded DRN neurons the spike rate, regularity and waveform width of each neuron was calculated and used as input to a clustering algorithm (see Methods). Clustering revealed neurons with one of three principal firing properties; a slow and regular firing pattern and broad spike waveforms, a slow and irregular firing pattern with

narrow spike waveforms, and fast-firing neurons with narrow spike waveforms (hereafter referred to as SR, SIR and FF neurons, respectively; Figure 2.2 – C). Silicon probes enabled the simultaneous recording of both multiple SR neurons as well as multiple SR neurons with neighboring FF and SIR neurons (Table 2.2).

The electrophysiological characteristics of the dataset of silicon probe-recorded DRN neurons were compared to a second dataset of 131 DRN neurons collected under the same conditions but using single unit glass micropipette recordings. The glass micropipette dataset contained SR, SIR and FF neurons whose firing rate, regularity and spike waveform width were very similar to corresponding neurons of the silicon probe dataset (Figure 2.2 – C, D). A chi-square test was conducted to determine if there was a significant association between neuron type and the two datasets. The chi-square statistic was calculated to be 4.6 with 2 degrees of freedom ($p > 0.05$) which indicated that the relative frequency of neuron types did not differ significantly between datasets.

2.3.3 Volatility of electrophysiological characteristics

Silicon probes enabled the firing activity of the same set of neurons to be recorded over long periods of time (Figure 2.3– A&B). Because visual inspection of spike trains over time indicated that the electrophysiological properties of neurons could fluctuate, an exploratory analysis was performed to quantify the volatility of each neuron's firing rate and regularity (see Methods).

For both firing rate and regularity, the degree of volatility varied across neuron types (main effect of neuron type on volatility of regularity – $F(2) = 4.1$, $p < 0.05$; rate – F

(2) = 4.4, $p < 0.05$), indicating that the electrophysiological properties of some neuron types were more stable than others.

Although SR neurons were overall slow and regular firing (Table 2.1), many neurons underwent periods of irregular firing (Figure 2.3). Similarly, though SIR neurons were generally slow firing (Table 2.1) many neurons underwent periods of elevated activity, and their firing rates were considerably more volatile than SR and FF neurons (Tukey t-tests, SIR-SR – $t = 3.1$ $p < 0.05$, SIR-FF – $t = 3.2$, $p < 0.05$). Relative to SR and SIR neurons, the electrophysiological properties of FF neurons were relatively stable over time. The firing rates of these neurons were less volatile than SIR neurons (see above) and their firing regularity was less volatile than both SR and SIR neurons (Tukey t-tests, FF-SR – $t = -4.4$, $p < 0.05$, FF-SIR – $t = -4.5$, $p < 0.05$).

2.3.4 Effects of acute citalopram administration on silicon probe-recorded DRN neurons

Experiments were performed to determine whether silicon-probe recorded DRN neurons were inhibited by the SSRI citalopram via a 5-HT_{1A}-dependent mechanism, and to test whether SR neurons were particularly affected relative to SIR and FF neurons. Mice received an initial acute injection of either saline or citalopram followed by a second injection with the 5-HT_{1A}-antagonist WAY100635.

The effect of saline and citalopram on the firing rate of DRN neurons was analyzed using a linear mixed-effects model, with neuron ID as a random effect (see Methods; Figure 2.4 – A). There was a significant three-way interaction between block (pre-versus post-drug administration), group (saline or citalopram) and neuron type (SR, SIR, or FF; $F(2) = 4.98$, $p < 0.05$). This indicated that drug administration affected

the firing activity of DRN neurons and did so differently depending on the drug treatment and neuron type.

SR neurons were inhibited by citalopram as evidenced by these neurons having lower firing rates post-drug compared to saline-treated controls (Figure 2.4– D, t ratio (7006) = 4.564, $p < 0.05$). Interestingly, analysis of the responses of individual SR neurons revealed a sub-population whose firing rates were little affected by citalopram (Figure 2.4– F). FF neurons were also inhibited by citalopram, though to a lesser extent than SR neurons (Figure 2.4-D, t ratio (7006) = 3.118, $p < 0.05$). The firing activity of SIR neurons was not affected by citalopram. Thus, there was no significant difference between the firing rate of these neurons pre- and post-drug by group (t ratio (7006) = 0.143, $p > 0.05$).

A similar mixed modeling approach was used to determine the effect of WAY100365 on the firing activity of DRN neurons. There was a significant three-way interaction between block (pre- versus post-WAY100365 administration), group (prior administration of saline or citalopram) and neuron type (SR, SIR, or FF). This indicated that WAY100365 administration affected the firing activity of neurons and did so differently depending on whether the neurons were recorded in mice previously administered citalopram or saline, as well as the neuron type in question ($F(2) = 24.687$, $p < 0.05$).

The firing rate of SR neurons increased following WAY100365 administration to citalopram- but not saline-treated mice (post – pre; CIT: t ratio (5324) = 11.9, $p < 0.05$, SAL: t ratio (5324) = -0.199, $p > 0.05$). Although FF neurons were inhibited by citalopram alone, this effect was not reversed by subsequent WAY100365 administration (pre – post; CIT: t ratio (5324) = 1.22, $p > 0.05$, SAL: t ratio (5324) =

-0.188, $p > 0.05$). Similarly, the activity of SIR neurons was not significantly affected by WAY100365 in either group (pre – post; CIT: t ratio (5324) = -0.205, $p > 0.05$, SAL: t ratio (5324) = 0.198, $p > 0.05$).

Taken together, citalopram affected the firing rates of DRN neurons in a neuron type-specific manner. While SR and FF neurons were inhibited to differing degrees by citalopram, SIR neurons were not. Furthermore, following citalopram-induced inhibition of firing rate, neuron types had differential sensitivity to WAY 100365. In mice that had previously been administered citalopram, administration of WAY100365 increased the firing rate of SR but not FF neurons.

2.4 Discussion

2.4.1 Silicon probe recordings of DRN neurons

The current study applied silicon probes for the first time to record from DRN neurons *in vivo*. It was shown that these electrodes could be targeted to the DRN and generate large scale simultaneous recordings of DRN neurons. Thus, silicon probes monitored an average of ~25 DRN neurons simultaneously and generated a large dataset of nearly 600 neurons. This high neuronal yield of DRN neurons is in line with similar studies that have characterized silicon probe recordings in various rodent forebrain regions *in vivo* (Berényi et al., 2014; Bragin et al., 2000; Sales et al., 2021; Steinmetz et al., 2018; Ulyanova et al., 2019).

The current study extends previous *in vivo* electrophysiological studies of the DRN that have generally used single unit recording techniques such as glass micropipettes to monitor the activity of single neurons at a time [e.g., (Jacobs & Fornal, 1999; Sakai & Crochet, 2001; Schweimer & Ungless, 2010)]. However, a small number of recent

studies have applied multi-channel recording methods to the DRN (Cohen et al., 2015; Li et al., 2016b; Z. Liu et al., 2014; Ranade & Mainen, 2009; Waterhouse et al., 2004) with most using tetrodes which comprise bundles of wire electrodes (Buzsáki, 2004). The key advantage of silicon probes over tetrodes is their high density of recording sites on a single electrode shank (Steinmetz et al., 2018). This electrode design is optimal for targeting recordings to small brain structures such as the DRN (Michelsen et al., 2007).

As silicon probes generated large scale simultaneous recordings of DRN neurons, their application in electrophysiological studies of the DRN 5-HT system may confer several benefits. First, the large number of neurons recorded may enable investigations to be made into the diversity of electrophysiological properties among putative 5-HT neurons (Urai et al., 2021). Recent transcriptomic and neuroanatomical studies have identified considerable heterogeneity among 5-HT neurons in the DRN (Huang et al., 2019; Okaty et al., 2019; Ren et al., 2019; Weissbourd et al., 2014), but due in part to the low yield of the single unit recording technology that has typically been used in electrophysiological studies in this region, it is not known how this diversity is expressed in their firing activity.

Second, the high neuronal yield and neuron type agnostic nature of silicon probe recordings may facilitate electrophysiological studies to report on the properties of non-5-HT neurons. As outlined in Chapter 1, there is evidence from electrophysiological, neuroanatomical, and behavioral studies that non-5-HT neurons in the DRN may both influence the activity of local 5-HT neurons and act as projection neurons in their own right (Challis et al., 2013; Qi et al., 2014; Sharp et al., 2007; Varga et al., 2001, 2003), but, possibly due to the low neuronal yield of single

neuron recording techniques, many previous *in vivo* electrophysiological studies have not reported on their properties and targeted recordings to 5-HT neurons [e.g., (Chaput, de Montigny, et al., 1986; Chaput et al., 1991; Mansari et al., 2005; Wilkinson & Jacobs, 1988)].

Finally, the simultaneous nature of silicon probe recordings may enable electrophysiological studies to assess for the presence of emergent population dynamics in the DRN network which have been hypothesized to be important for neuronal computations, have recently been identified in other subcortical neuromodulatory nuclei, and require simultaneous neuronal recordings to be detected (Humphries, 2018; Saxena & Cunningham, 2019; Totah, 2018; Urai et al., 2021; Yuste, 2015).

2.4.2 Electrophysiological properties

The present study quantified the electrophysiological properties of silicon probe recorded neurons and compared them to a second dataset obtained using glass micro pipettes. Clustering of silicon probe recorded neurons by their electrophysiological properties revealed three predominant electrophysiological profiles: (1) slow and regular firing neurons with broad spike waveforms, (2) slow and irregular firing neurons without broad spike waveforms, and (3) fast-firing neurons with narrow spike waveforms (SR, SIR, and FF neurons, respectively). These findings are in line with previous *in vivo* electrophysiological studies that have reported on the predominant electrophysiological profiles of DRN neurons (Aghajanian et al., 1968; Allers & Sharp, 2003; Jacobs & Azmitia, 1992; Nakahama et al., 1981; Sakai & Crochet, 2001; Schweimer et al., 2011; Schweimer & Ungless, 2010).

The relative frequency of DRN neuron types was very similar in the silicon probe and glass pipette datasets. This indicates that the electrophysiological properties of DRN neurons were not influenced by the recording method. This is important because there is evidence that electrophysiological properties can be influenced by recording parameters that may be affected by electrode design (Fiáth et al., 2021; Gold et al., 2006).

There is good evidence to support the contention that neurons with SR, SIR, and FF electrophysiological profiles match up with some of the main chemically defined neuron types in the DRN, and not least 5-HT neurons. In particular, there is strong evidence that SR neurons are likely to be a population of mostly 5-HT neurons. Previous *in vitro* electrophysiological studies that combined recordings with cell labelling and immunocytochemistry have reported that most DRN neurons with SR profiles were immunopositive for markers of 5-HT neurons (G. K. Aghajanian & Vandermaelen, 1982; G. Aghajanian & Vandermaelen, 1982). Furthermore, similar evidence has been reported more recently using *in vivo* juxtacellular labelling methods (Allers & Sharp, 2003; Hajós et al., 2007; Schweimer et al., 2011; Schweimer & Ungless, 2010).

There is also evidence from *in vivo* juxtacellular-labelling studies that FF neurons may be a population of mostly GABA neurons. Specifically, one study has reported that most DRN neurons with a FF profile were immunopositive for markers of GABA neurons (Allers & Sharp, 2003).

There is no similar evidence linking DRN neurons with SIR electrophysiological profiles to a single neurochemically defined neuron type. Instead, these neurons are likely to be a heterogeneous neuronal population of mostly non-5-HT neurons.

Indeed, two *in vivo* juxtacellular-labelling studies have reported that these neurons were generally immunonegative for markers of 5-HT neurons (Allers & Sharp, 2003; Schweimer & Ungless, 2010), with one study reporting that one SIR neuron was immunopositive for the catecholamine marker tyrosine hydroxylase (Schweimer & Ungless, 2010).

Some caution is warranted regarding the use of neuronal electrophysiological profiles to predict the neurochemical identity of DRN neurons. As outlined in Chapter 1, data obtained from studies combining *in vivo* electrophysiological recordings with cell labelling and histology indicate that neuron type classifications using electrophysiological properties may result in both false positive and false negative misclassifications. For example, juxtacellular labeling studies have identified, albeit rarely, SR neurons that were immunonegative for markers of 5-HT neurons and FF neurons that were immunopositive for markers of 5-HT neurons (Allers & Sharp, 2003; Kocsis et al., 2006; Schweimer & Ungless, 2010).

Despite this evidence for variation, we believe, for the following reasons, it is reasonable to interpret SR and FF neurons as being populations of mostly 5-HT and GABA neurons. First, as mentioned above, SR and FF neurons with unexpected immunopositivity for 5-HT and GABA markers have only been reported in small numbers. Second, immunoreactivity must always be interpreted with caution since antigen expression levels can be below the limit of detection. Third, as neuronal recordings in juxtacellular labelling studies typically have a short duration and as it was noted in the present study that DRN neurons had periods during which their firing rate and regularity deviated from typical levels, it is possible that the electrophysiological profile of neurons reported in juxtacellular studies that had

unexpected immunoreactivity may have been recorded during a period of unusual spiking activity.

2.4.3 Effects of the acute citalopram challenge on the spike rates of DRN neurons

The neurochemical identity of silicon-probe recorded DRN neurons was explored further using a pharmacological approach, that is inhibition in response to acute administration of the SSRI citalopram. As predicted from numerous previous studies (Gartside et al., 1995; Jacobs & Azmitia, 1992; Kirby et al., 2003; Scuvée-Moreau & Dresse, 1979; Sharp et al., 2007; Vandermaelen & Aghajanian, 1983), the firing rate of SR neurons was inhibited by citalopram and this effect was reversed by subsequent administration of the 5-HT_{1A} antagonist WAY100365. This latter finding indicates the involvement of 5-HT_{1A} autoreceptors which are well known to be expressed by large numbers of 5-HT neurons in the DRN (see below).

FF neurons were also inhibited by citalopram although to a lesser extent than SR neurons and in a manner that was not reversed by WAY100365. It is possible that some citalopram-sensitive FF neurons were 5-HT neurons. As noted above there is evidence from an electrophysiological and juxtacellular labeling study that a minority of FF neurons are immunopositive for 5-HT neuron markers (Allers & Sharp, 2003). On the other hand, the idea that some FF neurons are 5-HT neurons is not in keeping with the finding that the inhibitory effect of citalopram was not reversed by WAY100635. Alternatively, citalopram-sensitive FF neurons could be 5-HT-sensitive GABA neurons. Indeed, previous studies have identified that DRN GABA neurons express 5-HT_{2A/C} receptors and have increased c-fos expression following

administration of 5-HT₂ receptor agonists (Boothman et al., 2006; Boothman & Sharp, 2005).

As a group, SIR neurons were not inhibited by citalopram which further distinguishes them from SR and FF neurons and adds further support to the idea that these neurons are mostly non-5-HT neurons.

It should be noted that as with electrophysiological properties, caution should be taken when using the pharmacological properties of DRN neurons to classify their neurochemical identity. Thus, although histological and single cell sequencing studies have reported that 5-HT neurons differentially express 5-HT_{1A} receptors with respect to non-5-HT DRN neurons (Huang et al., 2019; Sotelo et al., 1990), *in vitro* electrophysiological studies have identified populations of 5-HT neurons that were not inhibited by 5-HT_{1A} agonists as well as non-5-HT DRN neurons that were (Beck et al., 2004; Kirby et al., 2003; Marinelli et al., 2004). Furthermore, single-cell transcriptomic analysis of DRN 5-HT neurons identified clusters of 5-HT neurons that express 5-HT_{1A} receptors to differing degrees, suggesting that subsets of 5-HT neurons may have differential sensitivity to SSRIs and 5-HT_{1A} receptor agonists (Huang et al., 2019; Ren et al., 2019). Nevertheless, given that most 5-HT neurons express 5-HT_{1A} receptors, and that 5-HT_{1A} sensitive non-5-HT neurons are generally reported to be rare, it is reasonable to interpret the sensitivity of SR neurons to 5-HT_{1A}-mediated inhibition by SSRIs as supporting them being mostly 5-HT neurons.

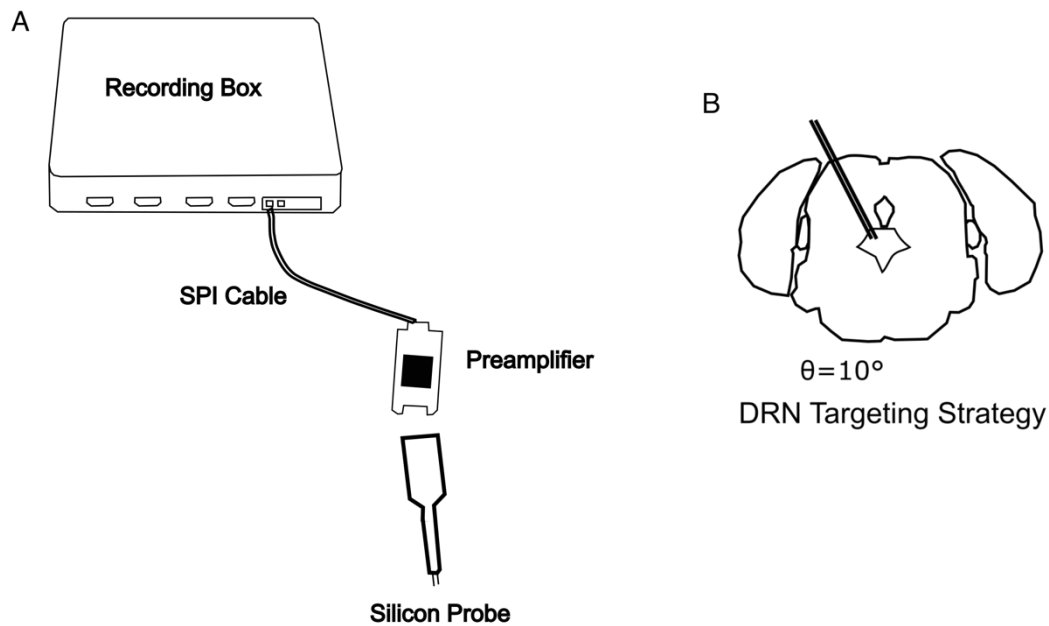
2.4.4 Conclusion

The present study utilized silicon probe electrodes to generate large-scale electrophysiological recordings of DRN neurons *in vivo*. The electrophysiological

properties of recorded DRN neurons were consistent with a second dataset obtained using traditional glass micropipettes. Three distinct neuron types were identified: SR, FF, and SIR neurons, with SR and FF neurons having electrophysiological properties consistent with those of 5-HT and GABA neurons reported in previous studies that combined single unit recordings with juxtacellular labelling. SR neurons were differentially inhibited by the SSRI citalopram, further supporting these neurons being a majority 5-HT neuron population. Experiments in the next chapter utilized the dataset to investigate the effects of an aversive foot-shock stimulus on the firing of DRN neurons.

2.5 Figures and tables

2.5.1 Methods figures



Methods Figure 2.1: DRN silicon probe recordings

- A)** Hardware used for silicon probe recordings. Analogue signals from the silicon probe electrode were converted to digital and multiplexed using an INTAN preamplifier before being streamed to the Open Ephys recording box via an INTAN SPI cable.
- B)** Strategy for targeting the DRN using silicon probes. The DRN is located underneath the sagittal sinus. In our experience, attempting to penetrate the blood vessel with the silicon probe can lead to blood loss and endanger the mouse. To circumvent this, the probe was inserted into the brain at a 10-degree angle.

2.5.2 Results figures

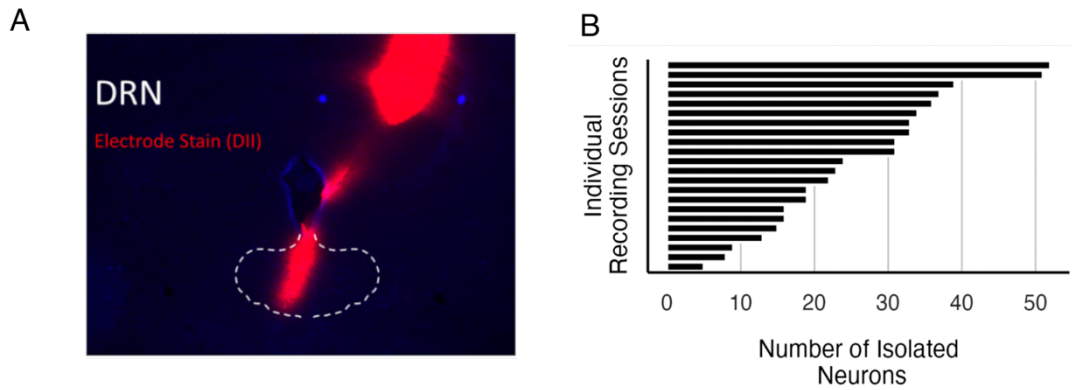


Figure 2.1: Silicon probe recordings of DRN neurons.

- A)** Histological image from an example mouse. Before being inserted into the brain, the silicon probe was coated in a lipophilic fluorescent dye DII. The red line of fluorescence starting at the brain surface (top right) and finishing at the DRN (white outline) suggests that the silicon probe was successfully targeted to the nucleus.
- A)** The number of simultaneously recorded neurons per recording.

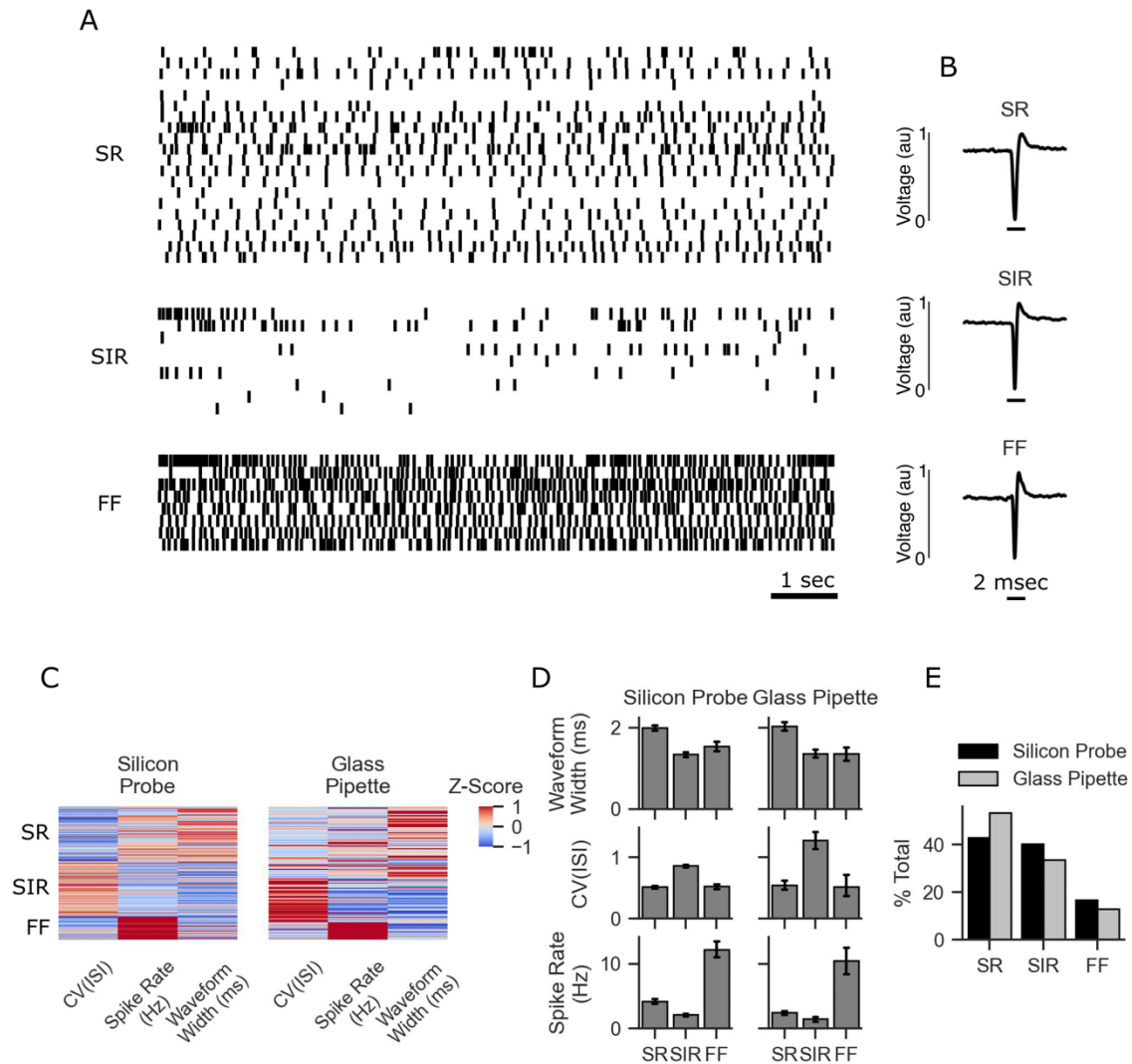


Figure 2.2: Identification of SR, SIR, and FF neurons.

- A)** Raster plot of 10 sec of spiking activity of all neurons (rows) recorded during a single recording session. Neurons are sorted vertically by their assigned neuron type (left key).
- B)** Average waveform for an example neuron of all three neuron types. The voltage of each waveform is 0-1 normalized. See 2 msec time scale bar below each waveform.
- C)** Visualization of the relative distribution of electrophysiological properties among the three neuron types in both datasets. Heatmaps display the spike regularity [CV(ISI)], firing rate and waveform width (time difference between spike initialization and repolarization; columns) of each neuron (rows) sorted by neuron type (vertically). Values are standardized for across metric comparisons (right

color key). In both datasets, SR neurons were slow and regular firing and had broad waveforms, SIR neurons were irregular firing with narrow waveforms and FF neurons were fast firing with narrow waveforms (see main text).

- D)** Comparison of electrophysiological properties (rows) across neuron types (x-axis) and datasets (column facets). Error bars cover $\pm$ SEM around mean.
- E)** Relative frequency of each neuron type in the silicon probe and glass pipette datasets.

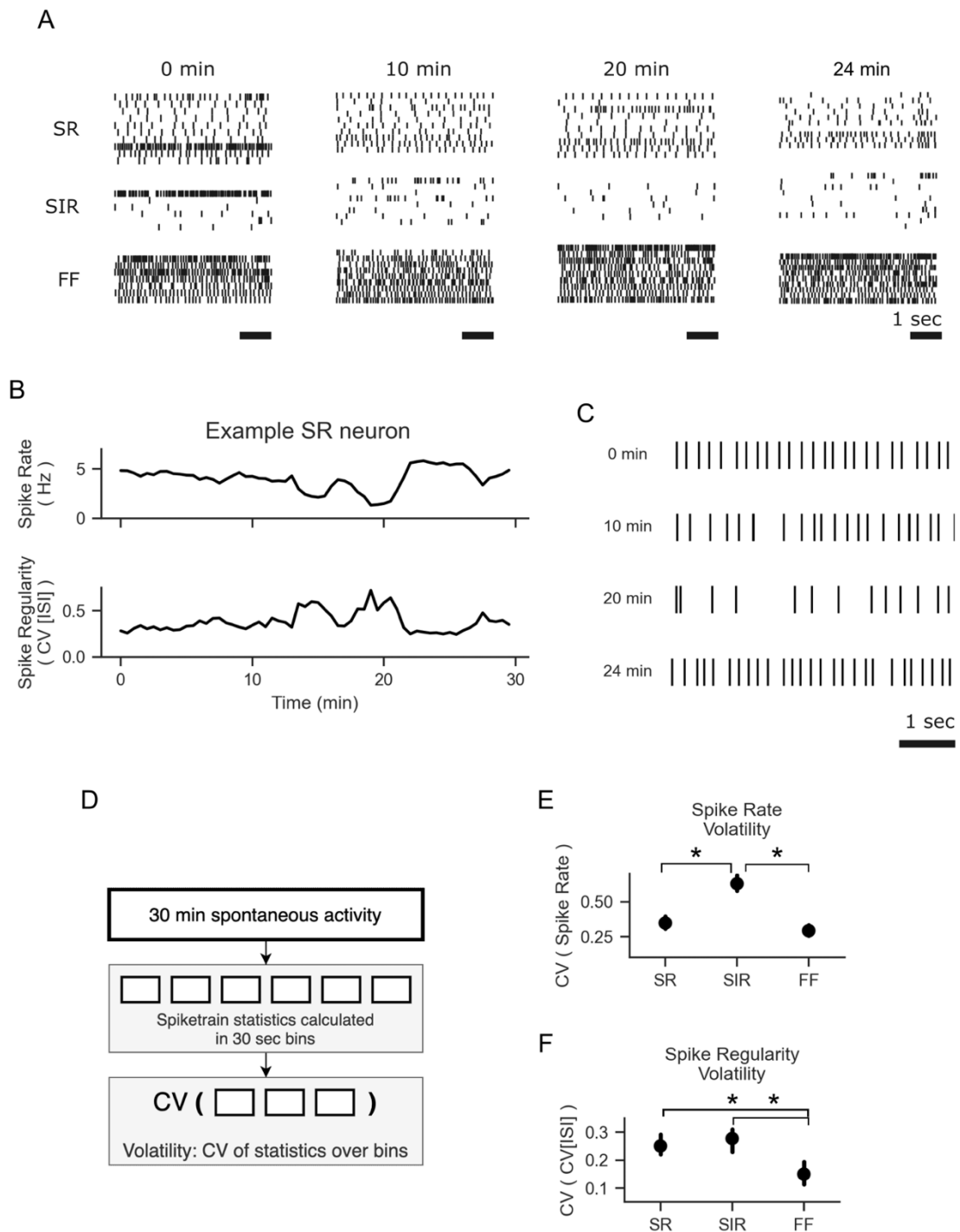


Figure 2.3: Variation of spike rate and spike regularity of DRN neurons over time

A) Raster plots of 5 secs of spiking activity of the same neurons at four different time-points (columns) within the same session. Neurons are ordered by neuron type (left key). The spike rate and regularity of neurons changed over time.

- B)** Change in spike rate (top) and regularity (bottom) of an example SR neuron
- C)** Raster plots showing 5-secs of spiking activity of the example neuron shown in C) at different points (rows) throughout the session
- D)** Schematic illustrating the procedure used to quantify volatility of spike rate and spike regularity. See methods for further details.
- E)** The spike rate of SIR neurons was more volatile than SR and FF neurons. Asterix indicate significant post hoc Tukey-adjusted t-test.
- F)** The spike regularity of FF neurons was less volatile than SR and SIR neurons. Asterix indicate significant post hoc Tukey-adjusted t-test.

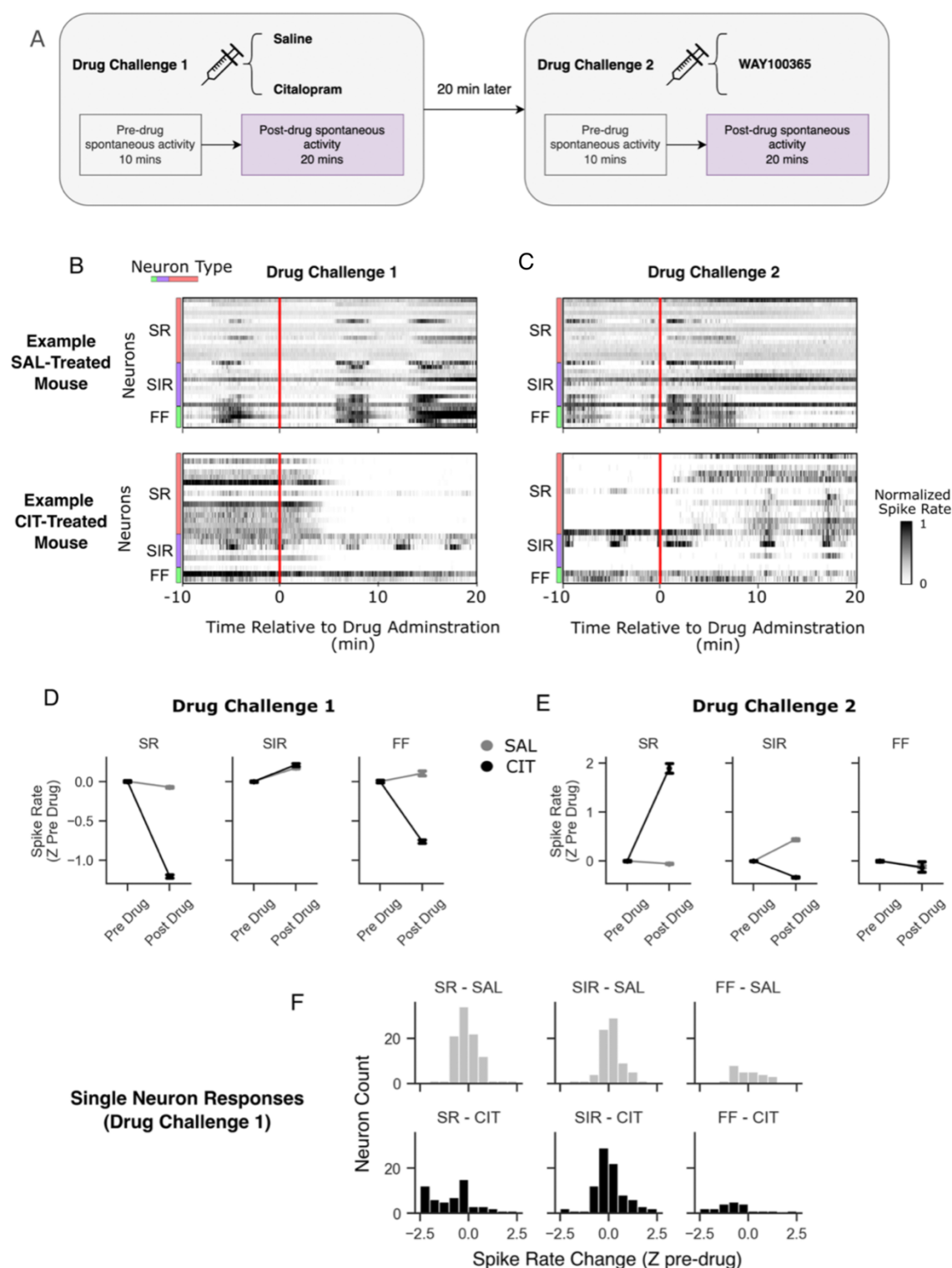


Figure 2.4: Effect of an acute citalopram challenge on the spike rate of DRN neurons.

A) Schematic illustrating the design of the pharmacological experiment. Mice received two drug challenges. For the first challenge, mice in one group were

administered saline and those in another group received citalopram. For the second challenge, all animals received the 5-HT_{1A}-agonist WAY100365. For both challenges, the spiking activity of each neuron during the 10-min period prior to drug administration was compared to that occurring 20 mins following drug administration.

- B)** Spiking activity of all neurons recorded in an example saline-treated mouse (top row) and citalopram-treated mouse (bottom row) around the first drug challenge. Neurons are ordered by neuron type (left color key). Spike rates are binned into 1 sec bins and normalized between 0-1 (see right color key).
- C)** Spiking activity of all neurons recorded in the example mice from **B** around the second drug challenge. Neurons are ordered by neuron type (left color key). Spike rates are binned into 1 sec bins and normalized between 0-1 (see right color key).
- D)** Effect of drug challenge 1 (citalopram or saline) on the spike rate of neuron types (column facets). Citalopram and not saline inhibited SR neurons. SR neurons were inhibited by citalopram to a greater extent than the other neuron types. Spike rate is Z-Scored to the pre-drug period to enable cross-neuron type comparisons.
- E)** Effect of drug challenge 2 (WAY100365) on the spike rate of neuron types (column facets), by drug received in the initial drug challenge (i.e., saline or citalopram). SR neurons recorded in animals which had previously received citalopram were activated by WAY100365, while those recorded in saline treated animals were not. SIR and FF neurons were not strongly modulated by WAY100365.
- F)** Neuron-level distribution of spike rate changes resulting from saline (top row) and citalopram (bottom row) administration by neuron type (columns). While most SR neurons were inhibited by citalopram, responses were heterogeneous.

2.5.3 Methods Tables

Apparatus	Purpose	Company
Microdrive	Control electrode depth	Inchworm
Amplifier	Amplify analogue electrode signal	Cygnus
Neurolog System	Bandpass filter between 300-500 Hz	Digitimer
Humbug	Remove 50 Hz mains noise	Quest Scientific
Micro 1401	Analog to digital conversion	Cambridge Electronic Design

Methods Table 1: Hardware for Glass Micropipette Recordings

2.5.4 Results Tables

Neuron Type	Spike Regularity [CV(ISI)]	Spike Rate [Hz]	Waveform Width [msec]
SR	0.56 ± 0.02	2.74 ± 0.15	1.99 ± 0.03
SIR	0.83 ± 0.01	1.73 ± 0.14	1.34 ± 0.03
FF	0.65 ± 0.03	9.63 ± 0.75	1.54 ± 0.06

Table 2.1: Electrophysiological Properties of Silicon Probe Recorded SR, SIR and FF Neurons.

Values are mean $\pm$ 2 SEM.

Neuron Type	Mean Count Per Recording	Proportion of Total Neurons (%)
SR	11.0 ± 1.5	43.78 ± 10.58
SIR	10.4 ± 1.8	38.15 ± 10.36
FF	4.7 ± 0.7	18.07 ± 8.20

Table 2.2: Frequency of DRN neuron types

Values are mean $\pm$ 2 SEM.

Chapter 3: Effects of an aversive stimulus on the activity of DRN neurons

3.1 Introduction

3.1.1 Role of the central 5-HT system in the processing of aversive stimuli

The central 5-HT system is linked to many brain functions ranging from modulating autonomic processes such as body temperature to regulating transitions between cognitive states (Dayan & Huys, 2009; Jacobs & Azmitia, 1992; Jacobs & Fornal, 1999). Among these many functions, the role of the 5-HT system in the processing of aversive stimuli and regulating negative emotional states is of particular interest. This is because dysregulation of the 5-HT system is associated with the pathogenesis of affective disorders such as anxiety and depression and their mainline drug treatment, selective serotonin reuptake inhibitors (SSRIs) (Cools et al., 2008; Cowen & Browning, 2015; Graeff et al., 1996; Marcinkiewicz et al., 2016).

Evidence for a role of 5-HT in the processing of aversive stimuli comes from many sources including neuroanatomical and behavioral studies. Neuroanatomical studies have demonstrated that 5-HT neurons in the DRN receive inputs from structures associated with aversion and nociception such as the nucleus raphe magnus and paraventricular thalamus and in turn project to regions associated with the processing of negative emotions such as the amygdala and medial prefrontal cortex (Dorocic et al., 2014; Michelsen et al., 2007; Petrov et al., 1994; Q. P. Wang & Nakai, 1994). Behavioral studies have demonstrated that 5-HT manipulations modulate

nociception and aversive behaviors (Marcinkiewicz et al., 2016; Oliveras et al., 1979; Ren et al., 2018; Sengupta & Holmes, 2019; Q. P. Wang & Nakai, 1994). For example, electrical stimulation of the DRN and drugs that enhance 5-HT neurotransmission reduced pain (Oliveras et al., 1979; Q. P. Wang & Nakai, 1994) and optogenetic activation of amygdala projecting DRN 5-HT neurons potentiated the acquisition of aversive fear memories from electric shocks (Sengupta & Holmes, 2019).

3.1.2 Effects of aversive stimuli on 5-HT neuron activity

There has been much research into the effects of aversive stimuli such as noxious electric shocks to the hind-paw or air puffs to the eye on the firing of 5-HT neurons in the DRN (Aghajanian et al., 1978; Cohen et al., 2015; Hayashi et al., 2015; Schweimer & Ungless, 2010). These experiments have demonstrated that aversive stimuli alter the activity of 5-HT neurons and that these effects can occur over multiple timescales. On fast timescales 5-HT neurons are reported to have short-lasting (50 – 300 msec) activations and inhibitions in response to the presentation of aversive stimuli (Cohen et al., 2015; Hayashi et al., 2015; Schweimer & Ungless, 2010). On slow timescales, the firing rate of 5-HT neurons has been reported to change during periods when aversive stimuli are repeatedly presented (Cohen et al., 2015; Hayashi et al., 2015). One interpretation of these results is that the firing activity of 5-HT neurons conveys multiple signals during aversive processing: that is, 5-HT neurons signal aversive state or expectations about future aversive stimuli on slow timescales and the presence of specific aversive stimuli on fast timescales (Cohen et al., 2015; Hayashi et al., 2015).

Most previous *in vivo* electrophysiological studies of the effects of noxious and aversive stimuli on the activity of DRN 5-HT neurons have used recording methods

that monitor the activity of one neuron at a time (Aghajanian et al., 1978; Cohen et al., 2015; Schweimer & Ungless, 2010). As reported in the previous chapter, silicon probes can generate *in vivo* large-scale recordings of DRN neurons from which the identity of 5-HT, GABA, and non-5-HT neurons can be estimated. The application of this approach to electrophysiological studies of the effect of aversive stimuli on DRN 5-HT neuron firing could confer several benefits as noted below.

3.1.3 Heterogeneity of DRN 5-HT neurons

Applying large-scale recording methods such as silicon probes to electrophysiological studies of the effects of aversive stimuli on DRN 5-HT neurons could enable these studies to investigate the diversity of sensitivity among 5-HT neurons. Although the central 5-HT system was originally hypothesized to transmit a unified signal (Jacobs & Azmitia, 1992; Jacobs & Fornal, 1999; Monti, 2010), recent molecular and anatomical analyses of DRN 5-HT neurons indicate that different subsets of neurons express different genes, receive different afferent input and project to different brain regions (Abrams et al., 2004; Huang et al., 2019; Okaty et al., 2019; Ren et al., 2019; Weissbourd et al., 2014). It is not known whether this diversity at the molecular level extends to the functional properties of these neurons.

Previous *in vivo* electrophysiological studies have reported diverse effects of aversive stimuli on DRN 5-HT neurons (Cohen et al., 2015; Schweimer & Ungless, 2010). For example, one study identified examples of 5-HT neurons that were activated, inhibited, and non-responsive to aversive foot-shocks (Schweimer & Ungless, 2010). However, this latter study, like most similar previous *in vivo* electrophysiological studies used single unit recording methods that yield low numbers of neurons and make it difficult to understand the heterogeneity on a quantitative level (Aghajanian

et al., 1978; Montagne-Clavel et al., 1995; Schweimer & Ungless, 2010; Urai et al., 2021).

3.1.4 Functional properties of DRN non-5-HT neurons

Large-scale recordings would also enable investigation of the effects of aversive stimuli on non-5-HT DRN neurons. As noted in Chapter 1, DRN 5-HT neurons are embedded amongst many other neuron types including GABA, glutamate, dopamine, and peptide neurons (Belin et al., 1979; Gamrani et al., 1979; Kaneko et al., 1990; Michelsen et al., 2007). Currently, the role of these neurons in aversive processing is poorly understood. It has previously been proposed that these non-5-HT DRN neurons serve to both regulate 5-HT neurons and also contribute to brain functions independently (Cho et al., 2017; Qi et al., 2014; Varga et al., 2003). Interestingly, there is evidence that non-5-HT DRN neurons may contribute to the processing of aversive stimuli. For example, optogenetic activation of DRN GABA neurons inhibited the acquisition of social avoidance (Challis et al., 2013).

3.1.5 Population coding of aversive stimuli

Large-scale recordings could also be used to determine whether aversion-evoked changes in activity in the DRN network is consistent with neurons using a population coding scheme. Although not yet investigated for aversion and the DRN, large-scale recordings in other brain regions indicate that neurons can encode stimuli through their joint activity (Carrillo-Reid et al., 2019; Georgopoulos et al., 1986; Laubach et al., 2000; Nicolelis & Lebedev, 2009; Runyan et al., 2017; Saxena & Cunningham, 2019; Simon et al., 2006). Studies have investigated whether neuronal populations might engage in a population coding scheme by comparing the performance of stimulus decoders that use single neurons with those that use groups of neurons

(Georgopoulos et al., 1986; Runyan et al., 2017; Saxena & Cunningham, 2019; Simon et al., 2006). These studies have interpreted improved performance of multiple neuron decoders relative to single neuron decoders as evidence of the neuronal population using a population coding scheme.

3.1.6 Chapter aims

Experiments in this chapter investigated how the activity of neurons of the DRN 5-HT system were affected by an aversive foot shock stimulus. Urethane-anesthetized electrophysiological recordings of DRN neurons were made *in vivo* using silicon probes and the identity of putative 5-HT, GABA, and non-5-HT neurons were estimated using neuronal electrophysiological properties. The responses of neurons were then monitored to an aversive foot-shock stimulus.

The response profiles of all single neurons to the foot shock stimulus were computed in order to investigate (1) the diversity of responses among putative 5-HT neurons, and (2) the sensitivity of putative non-5-HT neurons. For each neuron, two responses were calculated: fast timescale modulations in firing time locked to shock onset and slow timescale changes in firing rate during a block of repeated foot-shock presentations.

Finally, to investigate whether DRN neurons might signal foot-shock status using a population coding scheme, the performance of single neuron and multiple neuron foot-shock decoders were compared.

3.2 Methods

3.2.1 Dataset

Work in this chapter further analyzes a subset ($n = 8$ mice) of the silicon probe dataset of Chapter 2. This dataset contains simultaneous multi neuron recordings of DRN neurons made in urethane anesthetized mice. Recording sessions consisted of an initial 30-min block during which spontaneous activity was recorded as well as three additional blocks aimed at capturing the changes in neuronal activity occurring in response to presentations of an aversive foot-shock stimulus (detailed below).

3.2.2 Silicon probe recordings

See Chapter 2 for details of the silicon probe implantation procedure, data processing of silicon probe signals and assignment of SR, SIR, and FF neurons.

3.2.3 Delivery of foot-shock

3.2.3.1 *Aversive stimulus*

The aversive stimulus used in this study was mild, aversive foot-shocks. Foot-shocks were used for two reasons. Firstly, they have been used in previous electrophysiological studies of the effects of aversive stimuli on DRN 5-HT neurons and other neuronal populations (Brischoux et al., 2009; Schweimer & Ungless, 2010). Secondly, they have been demonstrated to activate nociceptive receptors and pain-associated spinal cord fibers (Urch et al., 2003).

3.2.3.2 *Hardware*

A schematic of the hardware used to control foot-shock delivery is shown in Methods Figure 3.1. Foot-shock delivery was controlled by an Arduino-programmed using

custom software. The output of the Arduino was converted to mild aversive electric shocks by a foot-shock generator (A360 stimulus isolator; World Precision Instruments), the output wires of which were connected to the mouse hind-paw. The Arduino's output was also connected to an Open Ephys recording box by an Open Ephys IO interface card (Siegle et al., 2017). The Open Ephys recording box recorded the timestamps at which the Arduino, and therefore the foot-shock generator, was active. The Open Ephys box temporally synchronized the timestamps with the signal from the silicon probe.

3.2.3.3 Experimental design

To enable the detection of both fast and slow timescale effects of foot-shock on DRN neurons, neuronal activity was monitored during three experimental blocks: pre-shock, foot-shock, and post-shock. The duration of all blocks was 10-min. During the foot-shock block, shocks (2 msec pulse duration, 2 mA) were delivered at regular 2 sec intervals (a total of 300 foot-shocks). No foot-shocks were delivered during the pre-shock and post-shock blocks.

Slow timescale effects were defined as changes in firing rate occurring between experimental blocks. Two slow timescale effects were quantified: those occurring between pre-shock and foot-shock blocks, and those between pre-shock and post-shock blocks. Fast timescale effects were defined as changes in firing rate during a short time window following foot-shock onset.

3.2.4 Analysis of single neurons

3.2.4.1 *Slow timescale effects*

Slow timescale effects were analyzed statistically using random slopes and intercepts mixed effects models. A separate model was fit to data of each neuron type, and each was parameterized in the same way. The dependent variable was spike counts of a neuron in a 30 sec time bin with the fixed effect being block (pre-shock, foot-shock, or post-shock) and random effects being block by neuron ID. The fixed effect modelled the overall effect of block across neurons, the random effect accounted for neuron variability in the effect of block. The significance of fixed effects was computed using F-tests and those of random effects were computed using likelihood ratio tests. The models were computed using the ‘lme4’ R package and effects were tested using the ‘LmerTest’ R package (Bates et al., 2015; Kuznetsova et al., 2017).

The response profiles of individual neurons were computed using 1-way ANOVAs with spike counts as the dependent variable and block (pre-shock, foot-shock, or post-shock) as the independent variable. Benjamini-Hochberg-adjusted F-tests were used to determine the significance of block. For neurons having a significant effect of block, Tukey *post hoc* t-tests were used to compare mean spike counts by block. The ‘pingouin’ python package was used to fit ANOVAs and perform *post-hoc* tests (Vallat & Raphael Vallat, 2018).

3.2.4.2 *Fast timescale effects*

Fast timescale effects were analyzed using random slopes and intercepts mixed effects models. A separate model was fit to data for each neuron type with each being parameterized in the same way. A peri-stimulus time histogram (PSTH) was constructed for each neuron as the summed spike counts occurring at latencies

between -0.5 to +1.5 sec following shock onset (0.01 sec bin width) over all trials. The dependent variable was then the spike counts of a neuron's PSTH during a time bin. The only fixed effect was window: whether or not the bin was during a 50 – 200 msec post shock window. Random effects were window by neuron ID. The fixed effect modelled the overall effect of window across neurons, and the random effect model accounted for neuron variability in the effect of window. The significance of fixed effects was computed using F-tests and that of random effects were computed using likelihood ratio tests.

The response profiles of individual neurons were computed using Mann-Whitney U-tests comparing PSTH spike counts occurring during the 50 – 200 msec window to those occurring at other offsets. P-values were adjusted for multiple comparisons using the Benjamini-Hochberg method. Mann-Whitney U-tests were computed using the 'SciPy' Python package (Virtanen et al., 2020).

3.2.4.3 Response profile stability

To determine whether the response profiles of neurons to foot-shocks were stable over time, the slow and fast timescale effects of each neuron were computed using data from either the first or second half of the foot-shock block and then the similarity of effects across session halves was calculated.

For slow timescale responses, firing rate during the entire pre-shock block was compared to data from either the first or last 150 trials of the foot-shock block. Neurons were classified as shock-inhibited, shock-activated, or not responsive using Mann-Whitney U-tests, and effects were computed as the difference between median pre-shock and foot-shock rates.

For fast timescale responses, PSTHs were constructed for each neuron using data from the first or second 150 foot-shock trials. Neurons were classified as shock-inhibited, shock-activated, or not responsive using Mann-Whitney U-tests comparing time bins occurring within a 50 – 200 msec post-shock window to those occurring at other offsets. Effects were computed as the difference between median in-window and out of-window firing rates.

For both slow and fast timescale effects, the overlap between first and second half response distributions was quantified using Spearman correlation of effects and pairs bootstrapped adjusted mutual information of response profiles. Spearman correlations were computed using the ‘SciPy’ Python package and adjusted mutual information was computed using the ‘scikit-learn’ python package (Pedregosa et al., 2012; Virtanen et al., 2020).

3.2.4.4 Cross-timescale response patterns

An analysis was performed to identify the most common cross-timescale foot-shock response profiles and detect associations between response patterns and neuron type. To detect cross-timescale response profiles neurons were clustered using their fast and slow timescale responses to foot-shock. Clustering was performed using the k-means algorithm ($k = 3$). The association of response profile and neuron type was determined using a Chi-square contingency test. Z-tests on standardized residuals were used as *post hoc* tests to identify over- and under-represented cells in a neuron type by response pattern contingency table. The ‘scikit-learn’ Python package implementation of k-means was used to cluster neurons and the ‘SciPy’ python package was used to perform the Chi-square analysis (Pedregosa et al., 2012; Virtanen et al., 2020).

3.2.5 Decoding analyses

3.2.5.1 General procedure

Decoders were trained to predict foot-shock status using DRN neuronal activity measurements. Different sets of decoders were trained and evaluated for analysis of fast and slow timescale effects of foot-shocks on neuronal activity. For fast timescale decoders, the target variable was whether changes in neuronal activity occurred during a 200 – 0 msec window prior to shock onset or a 50 – 200 msec window following shock onset. For slow timescale decoders, the target variable was whether changes in neuronal activity occurred during the pre-shock, foot-shock, or post-shock block. Decoders were trained using a 5-fold stratified sampling cross-validation routine. Their performance was evaluated by scoring predictions on the held-out data of each cross-validation fold using F1 scores and then taking the mean F1 score over folds. The classification algorithm used by decoders was light gradient-boosting machines, and the implementation used was that of the ‘LightGBM’ python package (Guolin Ke et al., 2017). Model fitting and evaluation was performed using the ‘scikit-learn’ python package (Pedregosa et al., 2012).

3.2.5.2 Decoder classes

To compare how the capacity of DRN neuronal activity to predict foot-shocks varied as a function of the number and types of neurons used, multiple classes of decoders were trained, and their performance was evaluated. The decoder classes were the following:

- **Single neuron decoders:** neuronal activity from a single neuron.

- **Single neuron shuffle decoders:** neuronal activity from a single neuron having had its correlation with foot-shock removed. For fast timescales this was achieved by shuffling inter-spike intervals prior to aligning to foot-shock onset. For slow timescales, spike count time-series were permuted following binning (1 sec bin width).
- **Population decoders:** neuronal activity from all neurons in a recording session.
- **Population shuffle decoders:** neuronal activity from all neurons in a recording session having had their correlation with foot-shock removed. This was achieved using the approach outlined for single neuron shuffle decoders.
- **Dropout decoders:** neuronal activity from all neurons in a recording session but with all neurons of one neuron type removed. For example, SR dropout decoders included all non-SR neurons from a recording session.
- **Limit decoders:** neuronal activity from a random subset of neurons from a single recording session. For example, a 5-neuron limit decoder used 5 randomly sampled neurons (without replacement) from a recording session.

3.2.5.3 *Comparison of decoder classes*

Mean F1 scores were compared using Wilcoxon rank sum tests. The paired variable was recording session (i.e., the performance of decoders of one class was compared with that of another class trained on the neuronal activity from the same recording session). Wilcoxon tests were computed using the ‘SciPy’ Python package (Virtanen et al., 2020).

3.2.6 **Software**

Data processing, transformations, and statistical analysis was performed using custom software written in R, Python, MATLAB, and SQL. All statistical analysis used

an alpha level of 0.05 and multiple comparisons were accounted for where appropriate using Tukey and Benjamini-Hochberg adjustments.

3.3 Results

3.3.1 Dataset

Silicon probe recordings were performed in urethane anaesthetized wild type mice ($n = 8$). Single neurons were isolated from electrode signals using the Kilosort2 spike sorting algorithm, yielding a total of 259 neurons (mean count per session: 32.38 ± 4.67).

In Chapter 2, the electrophysiological characteristics of these neurons were used to cluster them into three neuron types: slow regular, slow irregular and fast firing neurons (SR, SIR, and FF neurons; see Methods). A total of 111 SR, 118 SIR and 30 FF neurons were recorded (mean count per session: SR 13.88 ± 2.47 , SIR 14.75 ± 4.01 , FF 3.75 ± 1.06).

3.3.2 Slow timescale effects of foot-shock

Neuronal activity was recorded during three 10-min experimental blocks: pre-shock, foot-shock, and post-shock. During the foot-shock block a noxious foot-shock stimulus was applied every 2-sec for 10-min. No foot-shocks were applied during the pre-shock and post-shock blocks (Figure 3.1– A).

Averaged across neurons, the mean firing rate of neurons of all three neuron types was higher during foot-shock than pre-shock, and lower during post-shock than pre-shock (Figure 3.1– C). A mixed modelling approach was used to quantify such firing rate changes and determine whether responses were heterogeneous among neurons

(see Methods). For all three neuron types, there was a significant fixed effect of block, and additionally, the slope of block varied significantly across the levels of neuron ID (random slope of block). This indicated that the firing rates of each neuron type differed during the three blocks and that the effects of block were heterogeneous among neurons. For SR neurons, fixed effect of block: $F(2) = 19.16$, $p < 0.05$, significant random slope of block by neuron ID: $\chi^2(6) = 8542.8$; $p < 0.05$. For SIR neurons, fixed effect of block $F(2) = 19.24$; $p < 0.05$, random slope of block by neuron ID: $\chi^2(6) = 8242.3$; $p < 0.05$. For FF neurons, fixed effect of block: $F(2) = 5.043$; $p < 0.05$, significant random slope of block by neuron ID: $\chi^2(6) = 2675.8$; $p < 0.05$.

To further quantify the heterogeneity among neurons, the effects of block on the firing rate of each neuron was calculated using one-way ANOVAs and Tukey-adjusted t-tests. This analysis revealed the distribution of slow timescale foot-shock effects by neuron type (i.e., changes in firing rate from pre-shock to foot-shock; Figure 3.1– D). For SR neurons, $8.11 \pm 2.59\%$ were not modulated, $56.76 \pm 4.70\%$ were shock-activated and $35.14\% \pm 4.53$ were shock-inhibited. For SIR neurons, $9.32\% \pm 2.68$ were not modulated, $57.63 \pm 4.55\%$ were shock-activated and $33.05 \pm 4.33\%$ were shock-inhibited. For FF neurons, $10.00 \pm 5.48\%$ were not modulated, $43.33 \pm 9.05\%$ were shock-activated and $46.67 \pm 9.11\%$ were shock-inhibited.

This analysis also revealed the changes in firing rate between the pre-shock and post-shock blocks for each neuron (Figure 3.1– E). For SR neurons, $11.71 \pm 3.05\%$ were not modulated, $11.71 \pm 3.05\%$ were higher during post-shock, and $76.58 \pm 4.02\%$ were lower during post-shock. For SIR neurons, $15.25 \pm 3.31\%$ were not modulated, $16.10 \pm 3.38\%$ were higher during post-shock and $68.64 \pm 4.27\%$ were lower during post-

shock. For FF neurons, $13.33 \pm 6.21\%$ were not modulated, $23.33 \pm 7.72\%$ were higher during post-shock, and $63.33 \pm 8.80\%$ were lower during post-shock.

Taken together, these results demonstrated several findings. Firstly, slow timescale effects of foot-shock were present in SR neurons, but also SIR and FF neurons. Secondly, there was significant heterogeneity in slow timescale foot-shock effects on firing rate across neurons of all neuron types, with some being increased, some being decreased and some not responding. Thirdly, the firing rate of most DRN neurons was reduced during a period of spontaneous activity following a 10-min period of repeated foot-shock presentations.

3.3.3 Fast timescale effects of foot-shock

A similar mixed modeling approach was used to detect fast timescale effects of foot-shock on the firing rate of DRN neurons and to quantify the degree of heterogeneity among neurons (Figure 3.2). Briefly, PSTH spike counts were modelled using window as a fixed effect (whether the count occurred during a 50 – 200 msec post-shock window) and window by neuron ID as random effects. For SR neurons, there was no significant effect of window ($F(1) = 0.14, p > 0.05$) but a significant random effect of window varying across the levels of neuron ID (random slope of window by neuron ID; $\chi^2(2) = 2471, p < 0.05$). This indicated that there was no consistent fast timescale effect of foot-shocks across SR neurons but that the effect of window varied by neuron. Similarly, FF neurons showed heterogeneous responses: no significant fixed effect of window ($F(1) = 1.09, p > 0.05$) but a significant random slope of window by neuron ID random effect ($\chi^2(2) = 1170.2, p < 0.05$). For SIR neurons, there was both a significant fixed effect of window ($F(1) = 12.98, p < 0.05$) and a significant window by neuron ID random effect ($\chi^2(2) = 6746, p < 0.05$), indicating that these neurons

were modulated by foot-shocks on fast timescales in a consistent way across neurons, though effects did vary by neuron.

To further explore the degree of heterogeneity of fast timescale effects among DRN neurons, the response profile of each neuron was calculated. This was achieved by comparing median PSTH spike counts occurring inside and outside a 50 – 200ms post-shock window using Mann-Whitney U-tests (see Methods). This analysis revealed the distribution of fast timescale foot-shock effects by neuron type (Figure 3.2 – D). For SR neurons $35.14 \pm 4.53\%$ were not modulated, $25.23 \pm 4.12\%$ were shock-activated, and $39.64 \pm 4.64\%$ were shock-inhibited. For SIR neurons $33.90 \pm 4.36\%$ were not modulated, $49.15 \pm 4.60\%$ were shock-activated, and $16.95 \pm 3.45\%$ were shock-inhibited. For FF neurons: $33.33 \pm 8.61\%$ were not modulated, $33.33 \pm 8.61\%$ were shock-activated, and $33.33 \pm 8.61\%$ were shock-inhibited.

These results demonstrated that in addition to SR neurons the firing rates of SIR and FF neurons were modulated by foot-shocks on fast timescales. The degree of heterogeneity among neuron types was quantified, and this demonstrated that approximately equal proportions of SR and FF neurons were activated and inhibited by foot-shocks, and a greater proportion of SIR neurons were shock-activated relative to SR and FF neurons.

3.3.4 Stability of foot-shock response profiles

To determine whether the foot-shock response profiles of neurons were stable over time, the fast and slow timescale responses of each neuron were computed separately using data from the first and second halves of the foot-shock block, and then the similarity of response distributions was compared.

For slow timescale effects, firing rate changes from pre-shock to foot-shock blocks were computed separately for the first and second halves of the foot-shock block. The Spearman correlation coefficient of firing rate changes by session half was 0.85, $p < 0.05$. The adjusted mutual information of response classifications (i.e., not modulated, activated, inhibited) was 0.38, $p < 0.05$ (Figure 3.1– F).

For fast timescale effects PSTHs were constructed using either the first or last 150 trials, and spike counts occurring in a 50 – 200ms window post-shock were compared to those occurring at other offsets. The Spearman correlation coefficient for median count differences was 0.82, $p < 0.05$. The mutual information of response classifications was 0.41, $p < 0.05$ (Figure 3.2– G).

These results indicated that both the fast and slow timescale response profiles of DRN neurons to foot-shocks were stable over time.

3.3.5 Cross-timescale response patterns

To uncover the most common response patterns across slow and fast timescales, neurons were clustered using their slow and fast timescale response profiles (see Methods). The clustering analysis revealed three prominent cross-timescale response patterns: neurons activated on both slow and fast timescales, neurons activated on slow but inhibited on fast timescales and neurons inhibited on slow timescales and not responsive on fast timescales (Figure 3.2– H).

There was a significant association between neuron type and cross-timescale response pattern, indicating that some response patterns were over- and under-represented in some neuron types relative to others (Figure 3.2– I). Relative to SIR and FF neurons, a greater proportion of SR neurons were activated on slow timescales and inhibited

on fast timescales (Z-test on standardized Chi-square contingency residuals, $p < 0.05$). Relative to SR and FF neurons, a greater proportion of SIR neurons were activated on both fast- and slow timescales (Z-test on standardized Chi-square contingency residuals, $p < 0.05$).

3.3.6 Slow timescale decoding of foot-shock

To compare how the capacity of DRN neuronal activity to predict foot-shock block varied as a function of number and types of neurons, multiple classes of decoders were trained and evaluated. The performance of decoders predicting foot-shock block using the activity of single neurons alone (hereafter referred to as single neuron decoders) was higher than the performance of shuffle controls ($W = 10$, $p < 0.05$). However, the performance of decoders that used all neurons from a recording session (hereafter referred to as population decoders) was higher in all cases than the best single neuron decoder from that session ($W = 0$, $p < 0.05$; Figure 3.3– E). The improvement was not solely due to increased numbers of neurons as shuffled population decoders did not perform above chance levels ($t = -209.72$, $p < 0.05$; Figure 3.3– E). The performance of population decoders remained high even when all neurons of one neuron type were removed (hereafter referred to as dropout decoders; Figure 3.3– F).

The relationship between decoder performance and number of neurons included was analyzed by assessing the performance of decoders that used random subsets of available neurons (hereafter referred to as limit decoders). The performance of such limit decoders rapidly increased as the number of neurons used increased from 2 to 6 neurons, and saturated at approximately 12 neurons (Figure 3.3– G).

3.3.7 Fast timescale decoding of foot-shock

Decoders were trained to use shock-aligned neuronal data to predict whether spiking occurred during a pre-shock window (-200 – 0 msec) or a post-shock window (50 – 200 msec). Though the performance of single unit decoders was higher than shuffle controls ($W = 228$, $p < 0.05$), the performance of population decoders was higher than the best single unit decoder from the corresponding session ($W = 0$, $p < 0.05$; Figure 3.4– E). Population decoders of neuronal data whose alignment to foot-shock onset had been removed by shuffling inter-spike-intervals was not greater than chance ($t = -16.85$, $p < 0.05$; Figure 3.4– E). This demonstrated that the improvement of population decoders over single neuron decoders was not simply due to using more variables. The performance of all three dropout decoders was high (Figure 3.4– F), indicating that foot-shock status could be predicted even from remaining neurons even when all neurons of either SR, SIR or FF types were removed. The performance of limit decoders gradually increased as more neurons were included between 2 to 11 neurons, at which point it saturated (Figure 3.4– G).

3.4 Discussion

3.4.1 Large scale recordings of the effect of foot-shock on DRN neurons

A novel feature of the present study is that it quantified the effects of an aversive stimulus on the DRN 5-HT system using silicon probe electrodes to generate a dataset comprising many hundreds of neurons. In comparison, similar previous electrophysiological studies have used single neuron recording methods (Aghajanian et al., 1978; Hayashi et al., 2015; Schweimer & Ungless, 2010). The large dataset generated here enabled the heterogeneity of response profiles to be quantified across

different DRN neuron types. Whereas previous studies have targeted specific neuron types (mostly SR neurons), the current use of silicon probes allowed recording of large groups of neurons simultaneously in a way that was agnostic to the types of neurons recorded. This enabled the properties of putative 5-HT (SR) neurons to be quantified alongside putative non-5-HT (SIR and FF) neurons. It also eliminated a potential source of bias as the properties of individual neurons were not considered when selecting neurons for further analysis (Urai et al., 2021). Finally, because the firing activity of multiple neurons was recorded at the same time, it was possible to quantify the relationships between foot-shock-evoked responses and population activity.

3.4.2 Effect of foot-shocks on SR neurons

The firing rate of SR neurons was modulated by foot-shocks on multiple timescales. On slow timescales, the firing rate of SR neurons differed between pre-shock and foot-shock blocks whereas on fast timescales, the firing of SR neurons was modulated post-shock onset. These findings confirm previous *in vivo* electrophysiological studies that also identified effects of aversive stimuli on the activity of putative 5-HT neurons on both slow and fast timescales (Cohen et al., 2015; Hayashi et al., 2015). In the present study these effects were identified in anaesthetized mice whereas in previous studies animals were awake and engaged in complex behavioral tasks (Cohen et al., 2015; Hayashi et al., 2015). This is noteworthy because slow timescale modulations of 5-HT neuron activity have been interpreted to encode psychological or cognitive variables. For example, slow timescale changes in firing rate between blocks of aversive and appetitive stimuli were interpreted in one study as encoding expectations about future aversive stimuli (Cohen et al., 2015). As such cognitive processes may be reasonably expected to be absent in anaesthetized animals, the present data suggest that slow

timescale effects of aversive stimuli are more likely to be encoding other factors such as changes in brain state.

The slow and fast timescale response profile of each SR neuron to foot-shocks was computed and used to characterize the degree of responsivity across neurons and heterogeneity of responses. It was found that there were many SR neurons whose firing rate was activated, inhibited or non-responsive to foot-shocks on both timescales. In agreement with these findings, putative 5-HT neurons in the DRN have previously been reported to show varied response profiles to aversive stimuli (Cohen et al., 2015; Hayashi et al., 2015; Schweimer & Ungless, 2010). However, the current study is the first to quantify the degree of heterogeneity across a large population of putative 5-HT neurons.

The most common cross-timescale response patterns of SR neurons were identified by clustering fast- and slow timescale responses to foot-shocks. This approach demonstrated that many SR neurons have divergent responses across timescales as the most common cross-timescale response pattern was increased firing rate during the foot-shock block but inhibited firing rate during a short period following foot-shock onset. The presence of such divergent response profiles is consistent with slow and fast timescale effects of aversive stimuli being mediated by different mechanisms. One possible explanation is that fast and slow timescale effects of foot-shock are mediated by bottom-up and/or top-down input. The short latency of fast timescale responses suggests that they may be driven from ascending projections from nociceptive nuclei. Conversely, as the slow timescale effects were not coupled to stimulus onset, they could be mediated by top-down influences. The DRN receives afferent input from various nuclei involved in early nociceptive processing including

the nucleus raphe magnus as well as regions linked to top-down sensory and affective control such as the prefrontal cortex (Dorocic et al., 2014; Huda et al., 2020; Paneri & Gregoriou, 2017; Q. P. Wang & Nakai, 1994). The neural mechanisms underlying the shock-evoked changes in 5-HT neuron activity could be explored further by investigating the effects of manipulating higher order brain regions such as the prefrontal cortex on slow and fast timescale responses of SR neurons.

The response profiles of SR neurons to foot-shocks were heterogeneous and stable across time. One interpretation of this finding is that it supports the hypothesis of a differentiated 5-HT system: this envisages a collection of specialized 5-HT subsystems, each subsystem containing sets of 5-HT neurons that have common functional and molecular properties that affect brain processing in different ways to other 5-HT subsystems (Abrams et al., 2004; Okaty et al., 2019; Ren et al., 2018). In support of this idea, it has been reported that there are clusters of DRN 5-HT neurons that have overlapping gene expression profiles, patterns of afferent input, and projection targets (Abrams et al., 2004; Huang et al., 2019; Ren et al., 2019; Weissbourd et al., 2014). Furthermore, it has been reported that manipulations of the activity of subsets of 5-HT neurons can have different effects on behavior. For example, chemogenetic activation of amygdala-projecting 5-HT neurons was found to promote negative emotional states, while activation of PFC-projecting 5-HT neurons reduced helplessness (Ren et al., 2018). Future research could explore whether the SR neurons with different response profiles to aversive stimuli have different molecular properties or affect aversive behaviors in different ways when modulated.

Another interpretation of the current data is that SR neurons with different response profiles contribute to the same 5-HT population code. Thus, a downstream neuron

may interpret the signal from activated neurons differently given that inhibited neurons are not active. Such combinatorial coding has been shown by theoretical studies to have several advantages over coding schemes that use specialized neurons. In particular, the number of distinct stimuli that could be interpreted from the activity of a neuronal population is higher when combinatorial encoding mechanisms are used rather than sparse specialized encoding mechanisms (Fusi et al., 2016; Rigotti et al., 2013).

3.4.3 Effect of foot-shocks on FF and SIR neurons

Another important finding of the present study was that, in addition to SR neurons, the firing activities of both putative GABA (FF) and other non-5-HT (SIR) neurons were modulated by foot-shocks. Furthermore, it was found that FF and SIR neurons were modulated on both slow and fast timescales indicating that these neurons share the same complex cross-timescale response profiles to aversive stimuli as 5-HT neurons. These results expand on previous work that has either focused recordings to 5-HT neurons or not attempted to separate out DRN neurons by their neurochemical identity (Cohen et al., 2015; Hayashi et al., 2015) by quantifying how putative non-5-HT neurons are affected by an aversive stimulus and demonstrate that these neurons have widespread responsivity.

The response profiles of FF and SIR neurons could result from several scenarios. One is that these neurons receive afferent input and distribute it to principal 5-HT neurons. Consistent with this idea, anatomical and physiological studies have demonstrated that afferent input to the DRN can preferentially target GABA neurons which input to 5-HT neurons through a GABA receptor-mediated mechanism (Varga et al., 2001, 2003; Weissbourd et al., 2014). It is noteworthy that the most common

fast timescale response profile of SR neurons was inhibition, whereas that of SIR neurons was activation. The latter neurons could be responding to external input and thereby inhibiting local 5-HT neurons. The role of local non-5-HT neurons in shaping the response profiles of DRN 5-HT neurons could be explored further by determining how 5-HT neuron responses to aversive stimuli are affected by pharmacological or optogenetic manipulations of local non-5-HT DRN neurons.

Another scenario is that non-5-HT neurons do not contribute to aversive processing and their response profiles are a consequence of changes in 5-HT neuron activity. Consistent with this idea, 5-HT neurons have collateral projections throughout the DRN and non-5-HT neurons express 5-HT receptors and have been reported to have altered activity following manipulations of 5-HT neuron activity (Boothman et al., 2006; Boothman & Sharp, 2005). For example, one study demonstrated that DRN GABA neurons had increased c-fos expression following the administration of a 5-HT₂ receptor agonist (Boothman & Sharp, 2005).

A final scenario is that non-5-HT neurons could be principal neurons that project out of the DRN and that respond to aversive stimuli by effecting change at downstream brain structures. Consistent with this idea, non-5-HT DRN neurons project to regions associated with emotional control such as the VTA and PVN (Michelsen et al., 2007; Petrov et al., 1994; Qi et al., 2014). Furthermore, optogenetic activation of non-5-HT DRN neurons can affect affective behaviors. For example, activation of DRN GABA neurons inhibited the acquisition of social avoidance (Challis et al., 2013) and activation of DRN glutamate neurons modulated reward-seeking behaviors (Qi et al., 2014).

3.4.4 Comparison of single neuron and population decoders

Analysis of the performance of stimulus decoders demonstrated that, for both slow and fast timescales, the capacity of DRN neuronal activity to predict foot-shock status increased when more neurons were used to make predictions. This result demonstrates for the first time a relationship between an aversive stimulus and population activity in the DRN 5-HT system.

This evidence for improved performance of stimulus decoders with increasing numbers of DRN neurons is consistent with the findings of similar analyses in other brain regions including the primary motor and sensory cortex of rodents and monkeys (Georgopoulos et al., 1986; Laubach et al., 2000; Rigotti et al., 2013; Runyan et al., 2017). The latter studies have interpreted the results as supporting a population coding scheme, whereby downstream regions interpret the signal from the neuronal population from their joint activity (Rigotti et al., 2013; Saxena & Cunningham, 2019; Yuste, 2015). Supporting this conclusion, one study demonstrated that population stimulus decoders of motor cortical neurons in rats performed better than single neuron decoders and that behavioral performance correlated more strongly with the predictions of population decoders than those of single neuron decoders (Laubach et al., 2000). A similar approach could be used to further investigate whether neurons of the DRN 5-HT system encode aversive stimuli using a population coding scheme. For example, large-scale recordings could be made from DRN neurons as animals engaged in a behavioral task involving the learning of associations between a sensory and aversive stimulus.

Proof that the DRN 5-HT system uses a population code would have implications for current theories about the role of the 5-HT system in brain function. Various theories

of the role of 5-HT neurons in behaviors motivated by rewarding and aversive stimuli have been generated by recording single neurons during complex behavioral tasks and correlating the dynamics of individual neurons with behavioral outputs [e.g., (Cohen et al., 2015; Daw et al., 2002; Dayan & Huys, 2009; Miyazaki et al., 2012)]. For example, one study proposed a role for 5-HT neurons in patience for rewards by demonstrating that the firing rate of putative 5-HT neurons in the DRN was elevated whilst animals waited for an expected reward but not whilst waiting for an unrewarded stimulus (Miyazaki et al., 2012). However, if 5-HT neurons use a population code, then the activity of individual neurons may not necessarily correlate with any one behavioral/cognitive variable but instead contribute to a population representation (Rigotti et al., 2013; Saxena et al., 2021). Moreover, it has recently been demonstrated that population activity can be used to decode behaviors for which individual neurons do not have tuning (Saxena et al., 2021). Thus, using the properties of single 5-HT neurons to screen for physiological variables encoded in the activity of the DRN 5-HT system could lead to false negatives.

The presence of DRN 5-HT neurons using a population code does not necessarily argue against the hypothesis of a differentiated 5-HT system. Although it was found here that the performance of population decoders was higher than those of single neurons for both fast and slow timescales, improvements in performance were gained with a relatively small number of neurons (approximately 10). Therefore, the activity of subsets of 5-HT neurons with common projection targets could be read out using a population code even if the number of neurons within such subsets is small.

It is important to note that the mechanism by which DRN neurons represent an aversive stimulus or how that representation is read out by downstream neurons

cannot be established using decoding analyses alone (Ritchie et al., 2017). For example, neurons used by population decoders in the present study may have had different projection targets or may be interpreted by downstream neurons using a different algorithm than that used by the decoder.

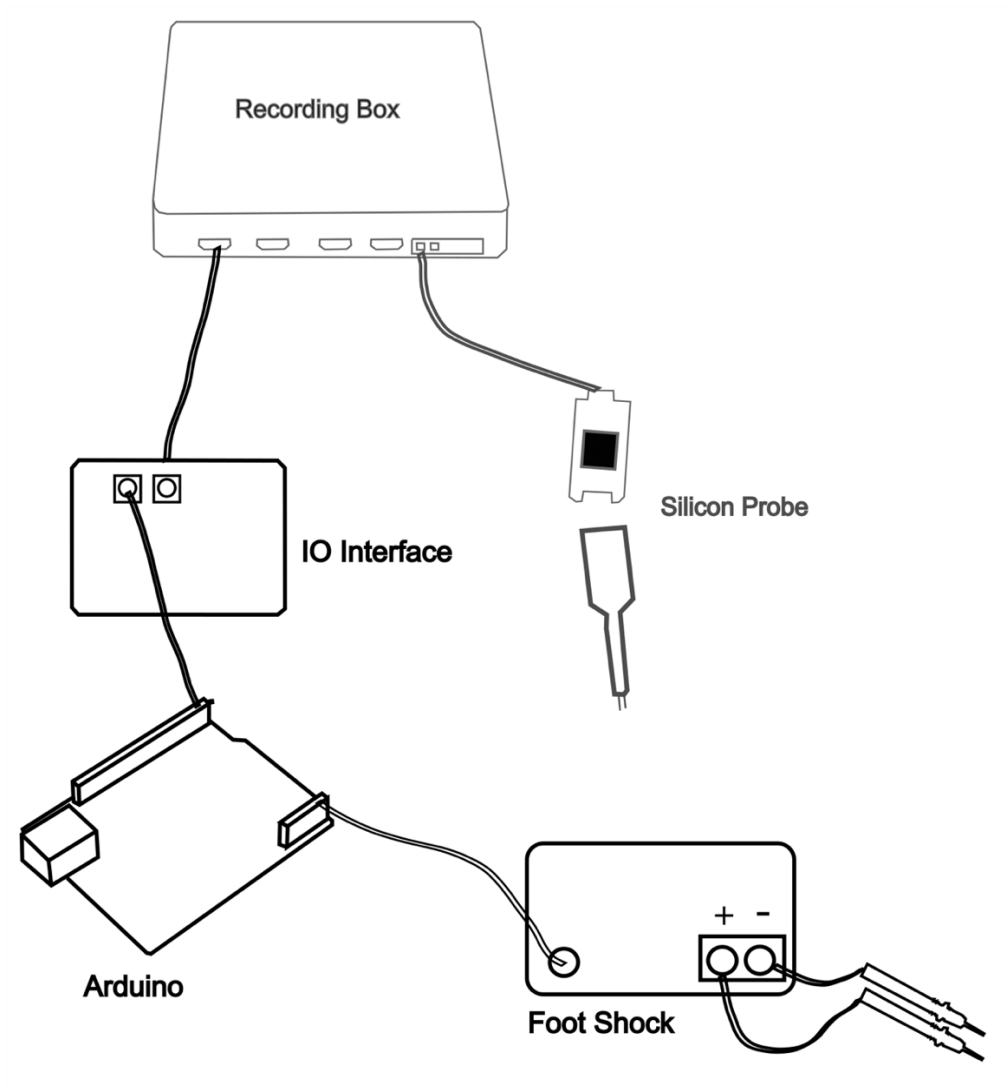
3.4.5 Conclusion

The present study used large-scale silicon probe recordings to quantify the effects of an aversive foot-shock stimulus on the activity of 5-HT and other neurons in the DRN network. It was demonstrated that the aversive stimulus had highly heterogeneous effects on the activity of putative 5-HT (SR) neurons on both slow and fast timescales. Moreover, the activity of many putative GABA (FF) and other non-5-HT (SIR) neurons were similarly modulated. Analysis of predictive models of foot-shock status demonstrated that performance improved when population rather than single neuron activity was used to make predictions.

Overall, this work (1) quantifies the diversity of responsivity among both 5-HT and non-5-HT DRN neurons to an aversive foot-shock stimulus and (2) provides initial evidence that neurons in the DRN network encode this stimulus using a population code.

3.5 Figures and tables

3.5.1 Methods Figures



Methods Figure 3.1: Hardware for foot-shock delivery.

Delivery of mild aversive foot-shock was controlled by an Arduino with output to a foot shock generator connected to the hind paw of the mouse. An Open Ephys recording box logged the timepoints at which the shocks occurred by recording the Arduino output via an IO interface card. Neuronal activity was recorded using a Cambridge Neurotech 32-channel silicon probe, connected to the Open Ephys recording box via an INTAN preamplifier and SPI cable.

3.5.2 Results Figures

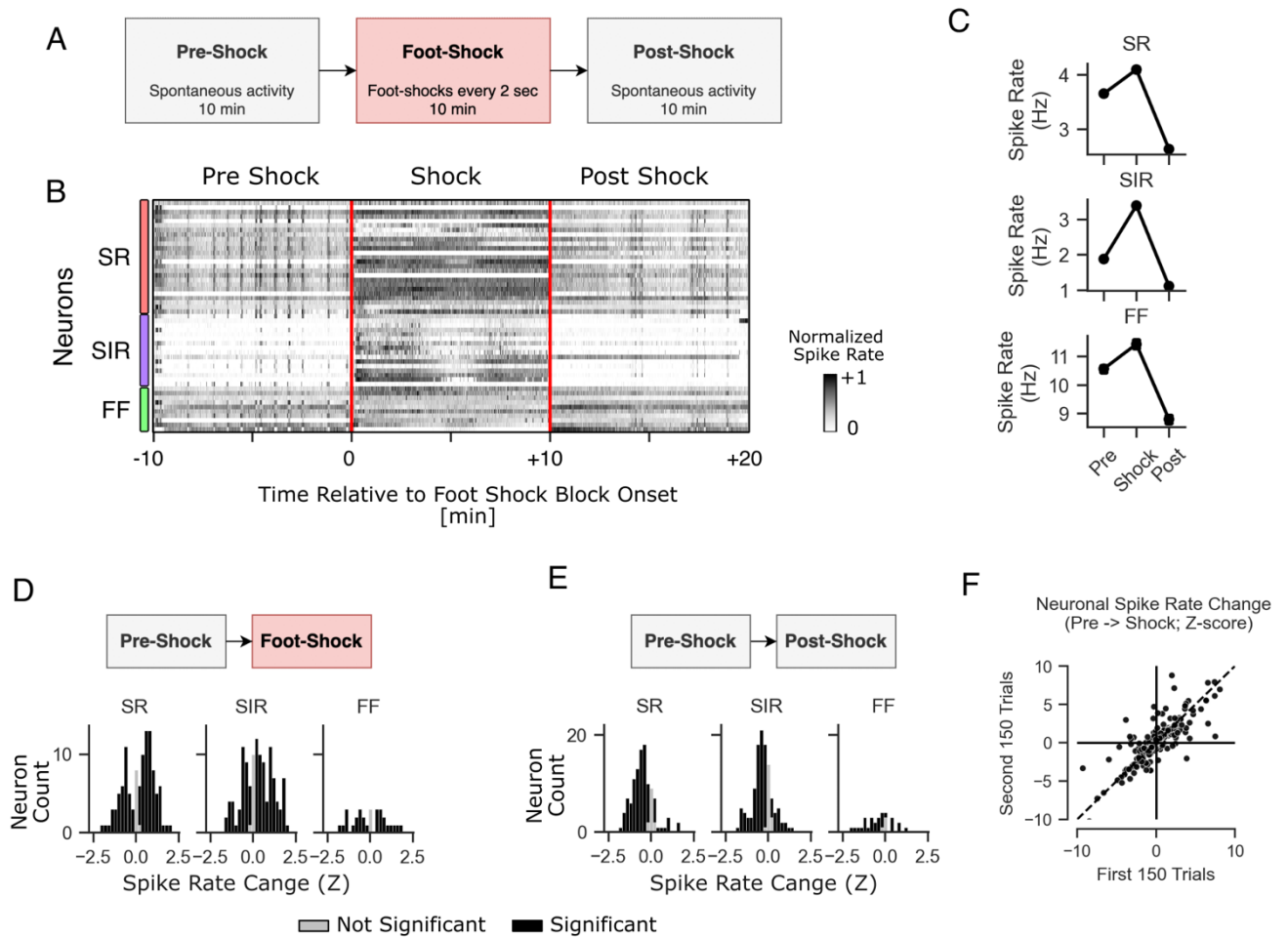


Figure 3.1: Slow timescale effects of foot-shock on DRN neuronal activity.

- A)** Illustration of the experimental design and the three experimental blocks: pre-shock, foot-shock and post-shock.
- B)** Example spike trains of all neurons recorded in a single recording session. Neurons are ordered by neuron type (see left key). Firing activity is smoothed using a Gaussian filter and normalized between 0 and 1 (for illustrative purposes only, not used in statistical analyses).
- C)** Mean firing rate (y-axis) of neurons in each block (x-axis) by neuron type (row-facets). Error bars are 95% confidence interval estimates around the mean.
- D)** Distribution of slow timescale foot-shock effects across neurons according to neuron type (column facets). A negative value indicates the neuron was less active

during the foot-shock block compared to the pre-shock block. Significant changes are indicated by color (see bottom color key).

- E)** Distribution of slow timescale post-shock effects across neurons according to neuron type (column facets). A negative value indicates the neuron was less active during the post-shock block compared to the pre-shock block. Significant changes are indicated by color (see bottom color key).
- F)** Stability of slow timescale responses over time. Distribution of slow timescale foot-shock effects when calculated exclusively using the first (x-axis) and last (y-axis) 150 foot-shock trials. One point is plotted for each neuron. Stable responses over time are indicated by points appearing on the diagonal.

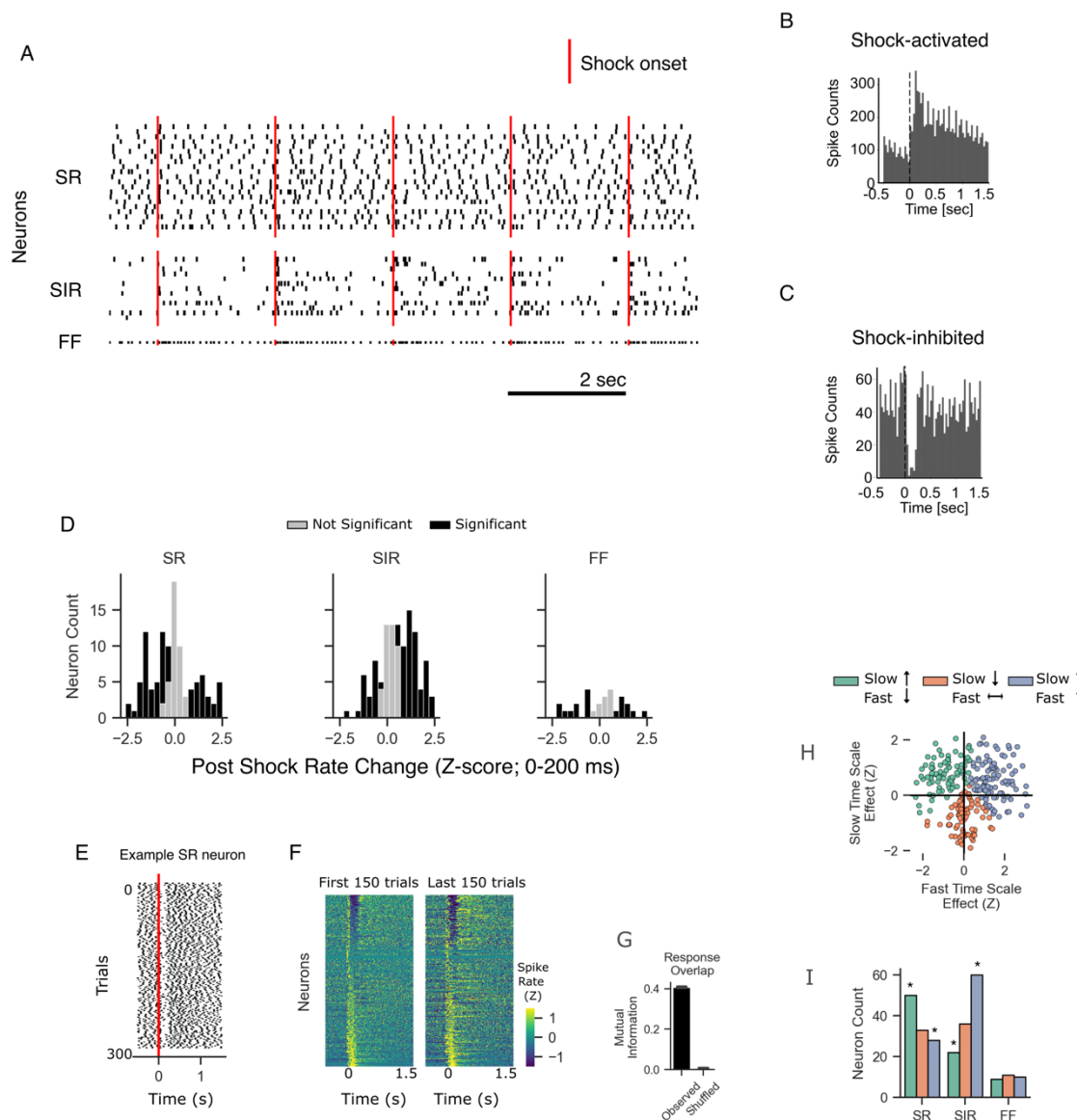


Figure 3.2: Fast timescale effects of foot-shock on DRN neuronal activity.

- A)** Example of a raster plot showing the firing activity of all neurons recorded in a single recording session during the presentation of foot-shocks (red lines). Neurons are ordered by neuron type (see left key).
- B)** Example PSTH of an SR neuron activated by foot-shock.
- C)** Example PSTH of an SR neuron inhibited by foot-shock.
- D)** Distribution of fast timescale responses across neurons according to neuron type (column facets). Negative values indicate that the neuron was less active in a 0 -

200 msec window following shock-onset compared to other timepoints. Significant differences are denoted by color (see top color key).

- E)** Example raster plot showing the firing activity of an inhibited SR neuron during all 300 trials (rows). The neuron response was constant over time, though spike counts following shock onset varied over trials.
- F)** Stability of fast timescale responses. Heatmap showing standardized PSTHs of all neurons calculated during the first (left column) and last (right column) 150 foot-shock trials. Each row is the PSTH of one neuron, with the two plots have the same neuronal ordering.
- G)** Overlap (adjusted mutual information) of fast foot-shock response classifications (inhibited, activated and no response) computed during the first and second halves of the foot-shock block.
- H)** Fast and slow timescale effects of foot-shock (x and y-axes, respectively) of each DRN neuron colored according to their cross-timescale response pattern (top color key). Examples of neurons activated on slow and on fast timescales (purple), activated on slow timescales and inhibited on fast timescales (green), and inhibited on slow timescales and not responsive on fast timescales (orange).
- I)** Distribution of foot-shock response patterns according to DRN neuron type. Slow timescale activated, and fast timescale inhibited neurons are over-represented in SR neurons with respect to other neuron types, and slow and fast timescale activated neurons were overrepresented among SIR neurons. `\*` indicate significant *post hoc* test of standardized residuals following Chi square contingency test.

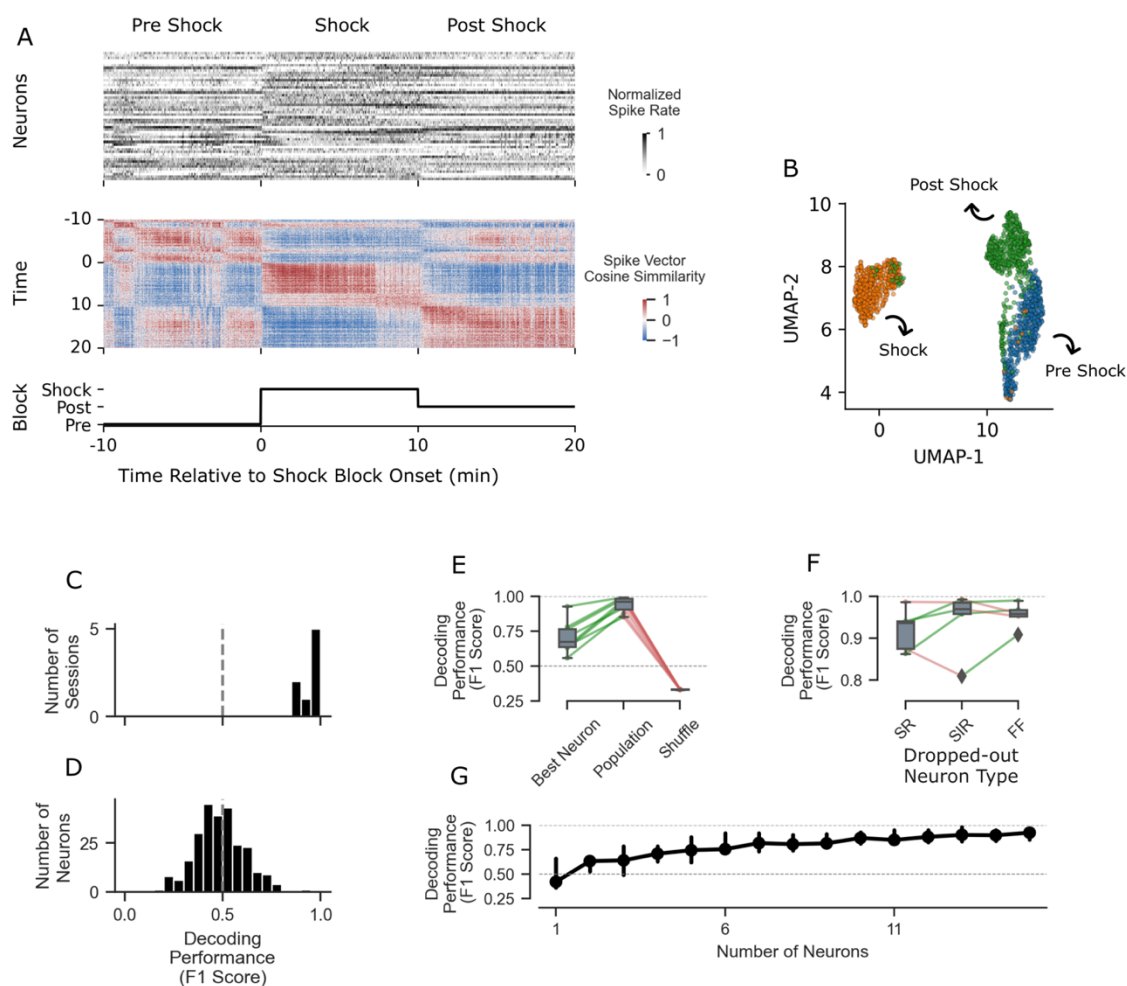


Figure 3.3: Slow timescale decoding of foot-shock from DRN neuronal activity.

- A)** Activity of DRN neurons from one example session during the pre-shock, foot-shock, and post-shock blocks. Top: activity of all neurons, normalized between 0 and 1. Middle: Similarity map of DRN population activity at all timepoints. Time is represented on x and y axes and the color indicates the normalized cosine similarity of spike count vectors at corresponding timepoints. Bottom: Indicator for experimental block.
- B)** UMAP decomposition of neuronal activity. Points are timepoints colored according to experimental block (see labels). The decomposition was performed on a (time by neurons) matrix over the neurons dimension. The data is from the recording shown in **A**. The two plots are shown to demonstrate visually that

experimental blocks are associated with discriminable patterns of population activity.

- C)** Distribution of F1-scores of experimental-block decoders trained using population activity (one decoder per recording).
- D)** Distribution of F1-scores of experimental-block decoders trained using single neurons (one decoder per neuron).
- E)** Performance of decoders by decoder class. Each decoder trained using population activity performed better than the best single neuron from each session. Decoders trained using shuffled population data did not performed above chance.
- F)** Performance of neuron type dropout decoders (i.e., decoders trained on population activity with all neurons of a specific neuron type having been removed).
- G)** Performance of limit decoders (i.e., decoders trains on random subset of neurons of a specific size).

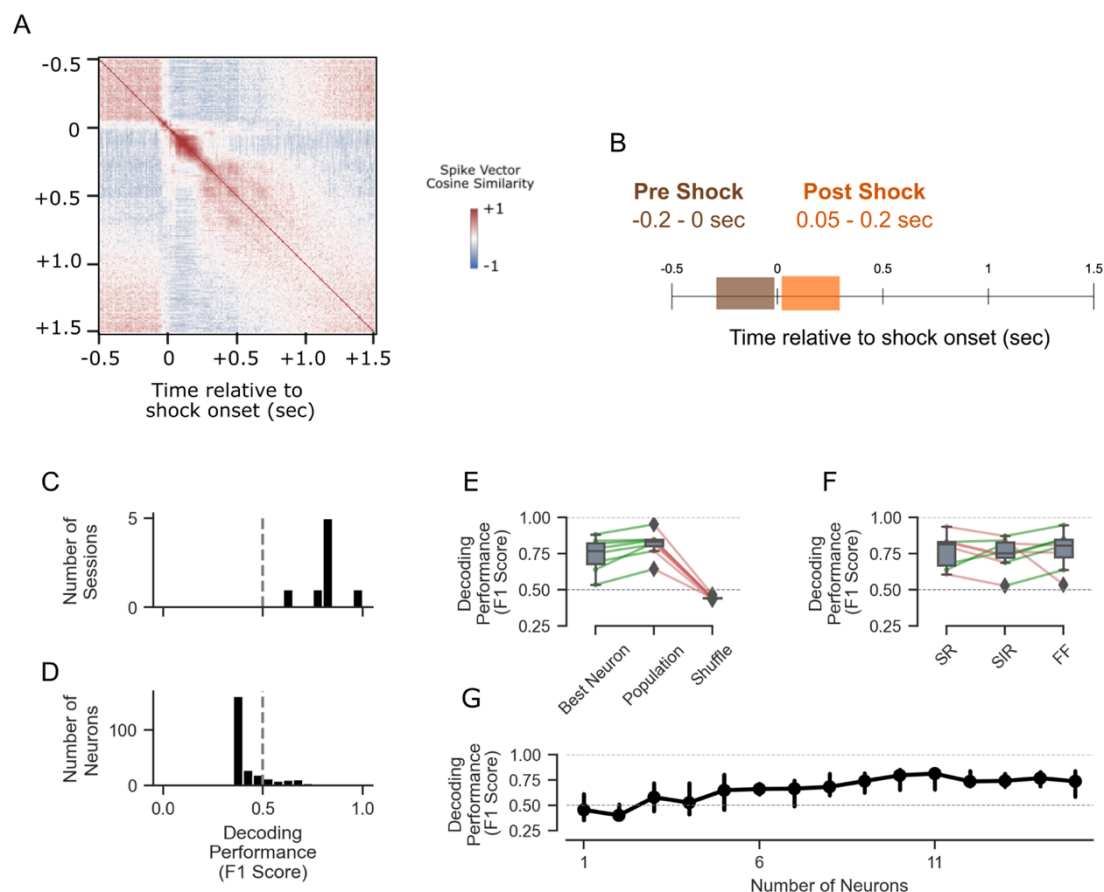


Figure 3.4: Fast timescale decoding of foot-shock status from DRN neuronal activity.

- A)** Similarity map of DRN population activity at temporal offsets relative to foot-shock onset. Time is represented on x and y axes and the color indicates the normalized cosine similarity of spike count vectors at corresponding timepoints.
- B)** Schematic illustrating the decoding objective: neuronal activity was used to predict whether timepoints occurred in a pre-shock or post-shock window.
- C)** Distribution of F1-scores of decoders trained using population activity (one decoder per recording).
- D)** Distribution of F1-scores of decoders trained using single neurons (one decoder per neuron).
- E)** The performance of decoders by decoder class. Each decoder trained using population activity performed better than the best single neuron from each

session. Decoders trained using shuffled population data did not perform above chance (dashed line).

- F)** Performance of neuron type dropout decoders (i.e., decoders trained on population activity with all neurons of a specific neuron type having been removed; see Methods).
- G)** Performance of limit decoders (i.e., decoders trains on random subset of neurons of a specific size).

Chapter 4: Quantification of neuronal interactions within the DRN network

4.1 Introduction

4.1.1 Interactions between DRN 5-HT neurons

There has been a significant research effort to characterize the properties of DRN 5-HT neurons and the factors that regulate their activity (Jacobs & Azmitia, 1992; Okaty et al., 2019; Sharp et al., 2007). One aspect of the dynamics of 5-HT neurons that remains poorly understood is the way in which their activity is influenced by other neurons in the DRN network. The DRN is a highly heterogeneous brain region. 5-HT neurons comprise approximately 30 – 50% of neurons and are embedded amongst glutamate, GABA, dopamine, and peptide neurons (Baker et al., 1990, 1991; Descarries et al., 1982; Kaneko et al., 1990; Lu et al., 2006; Wiklund et al., 1981). Moreover, the findings of recent neuroanatomical and transcriptomic studies indicate that there are multiple subclasses of 5-HT neurons with differing afferent input patterns, projection targets and gene expression profiles (Huang et al., 2019; Okaty et al., 2019; Ren et al., 2018, 2019). It is not known how this diversity at the molecular level is expressed in the dynamics of DRN neurons.

The relationships between the dynamics of neurons within a neuronal population have been quantified in other brain regions (Carillo-Reid & Yuste, 2020; Cohen & Kohn, 2011; Humphries, 2018; Totah, 2018). One approach has been to record from multiple neurons simultaneously using *in vivo* electrophysiology and quantify

statistical relationships between the spiking activity of neurons (Cohen & Kohn, 2011). This methodology has not yet been applied to the DRN 5-HT system, indeed most reports of DRN neuron activity have utilized methods that generate recordings of just one neuron at a time [e.g., (Jacobs & Fornal, 1999; Sakai, 2011; Schweimer & Ungless, 2010)].

Data in Chapter 2 demonstrated that silicon probe electrodes can record from multiple DRN neurons simultaneously and that the identity of 5-HT, GABA, and non-5-HT neurons can be estimated from these recordings. Silicon probes therefore have the potential to generate recordings that enable investigations to be made into the relationships between the dynamics of simultaneously recorded DRN neurons in a neuron type specific manner.

4.1.2 Coordinated activity among DRN 5-HT neurons

Although it is not known how the activity of 5-HT neurons are related to neighboring 5-HT neurons in the DRN network, the physiological properties of these neurons are consistent with their activity being coordinated in some way. Two factors can lead to neurons having correlated activity: neurons having common afferent input and neurons directly influencing each other's spiking activity via synaptic communication (Cohen & Kohn, 2011). There is evidence for DRN 5-HT neurons both having common input and directly influencing the activity of neighboring 5-HT neurons (Sharp et al., 2007; Weissbourd et al., 2014).

Neuroanatomical studies support 5-HT neurons having common patterns of afferent input. For example, cell-type specific tracing of the inputs of 5-HT neurons identified groups of neurons with overlapping input from multiple brain regions including the

prefrontal cortex and hypothalamus (Weissbourd et al., 2014). Such input may provide synchronous drive to these 5-HT neurons and coordinate their activity.

Neuroanatomical studies also indicate that 5-HT neurons may directly influence each other's activity. These studies have shown that 5-HT neurons send collateral projections throughout the DRN, receive synapses from other 5-HT neurons, and express 5-HT receptors including 5-HT_{1A/B}, 5-HT_{5B} and 5-HT₆ receptors (Barnes & Sharp, 1999; Brouard et al., 2015; Sharp et al., 2007; Sotelo et al., 1990; Weissbourd et al., 2014). This work is supported by studies that have used *in vivo* electrophysiological recordings combined with pharmacological manipulations to demonstrate that putative 5-HT neurons are sensitive to 5-HT and 5-HT receptor agonists (Brouard et al., 2015; Sharp et al., 2007; Vandermaelen & Aghajanian, 1983). For example, it has been commonly reported that most putative 5-HT neurons are inhibited when 5-HT is locally applied to the DRN (Vandermaelen & Aghajanian, 1983).

It is important to identify how activity is coordinated among 5-HT neurons because different activity structures could imply that the 5-HT system uses distinct coding mechanisms (Humphries, 2018). One important candidate structure is modular, whereby the activity of clusters of 5-HT neurons (i.e. neuronal ensembles) are correlated with each other but not necessarily with other clusters (Carillo-Reid & Yuste, 2020; Humphries, 2011, 2018). Modular neuronal dynamics have been identified in neuromodulatory systems, the noradrenergic system originating from the locus coeruleus (LC; Totah et al., 2018). Ensembles of noradrenergic neurons in the LC were reported to have common projection targets and electrophysiological properties (Shahryar Noei et al., 2022; Totah et al., 2018), and this was used as

evidence to argue that the LC noradrenergic system uses a differentiated coding scheme, a coding scheme in which different functions are performed by specific clusters of neurons (Totah et al., 2019). The DRN 5-HT system has been hypothesized to act in a similar differentiated manner [e.g., (Abrams et al., 2004; Okaty et al., 2019)]. Whether DRN neuron dynamics are consistent with this hypothesis could be assessed by determining whether correlations between these neurons have a modular structure.

4.1.3 Coordinated activity of 5-HT neurons and local non-5-HT neurons

The activity of DRN 5-HT neurons may be related to local non-5-HT neurons as there is evidence that they receive common inputs and can directly alter each other's spiking activity. Most studies have focused on the relationship between 5-HT and GABA neurons.

The capacity of DRN GABA neurons to influence local 5-HT neurons has been investigated by neuroanatomical and electrophysiological studies. Neuroanatomical studies have demonstrated that GABA neurons project throughout the DRN, including onto 5-HT neurons, and that 5-HT neurons express GABA receptors (Allers & Sharp, 2003; Boothman et al., 2006; Sharp et al., 2007; Weissbourd et al., 2014). Electrophysiological studies have demonstrated that putative 5-HT neurons are inhibited by local applications of GABA and GABA receptor agonists (Boothman et al., 2006; Judge et al., 2004; R. Liu et al., 2000).

Neuroanatomical studies also indicate that afferent input to the DRN may simultaneously target both 5-HT and non-5-HT neurons. For example, one study used cell-type specific transsynaptic tracing to quantify the extent to which DRN inputs

synapsed on 5-HT and GABA neurons (Weissbourd et al., 2014). It was found that excitatory input from several brain regions including the hypothalamus and medulla projected onto 5-HT and GABA neurons in similar proportions (Weissbourd et al., 2014). This indicates subsets of 5-HT and GABA neurons could have positively correlated activity driven by external afferent inputs.

Quantifying how the activities of 5-HT neurons are related to those of local GABA neurons could inform how the latter neurons influence the 5-HT system. For instance, one possibility is that GABA neurons exert a tonic inhibitory influence on neighboring 5-HT neurons, a relationship that would manifest as negative correlations between their spiking activities. Another possibility is that GABA neurons contribute to the coordination of activity across 5-HT neurons, for example, when a certain 5-HT ensemble is active, inhibiting non-ensemble 5-HT neurons. This relationship could present at the level of neuronal correlations as GABA neurons being positively correlated with a 5-HT ensemble and being negatively correlated with non-ensemble neurons.

4.1.4 Timescale of DRN correlations

Multiple temporal aspects of activity can be correlated across neurons (Bair et al., 2001; Kodama et al., 2018; Okun et al., 2019; Smith & Kohn, 2008). On fast timescales the spike timing of neurons can be synchronized whereas on slow timescales the spike rates of two neurons can co-fluctuate. Moreover, the pattern of neuronal correlation can differ across timescales. For example, cortical recordings identified diverse coupling of neurons to local population activity that differed across timescales: the neurons that were most strongly coupled on one timescale were not necessarily strongly coupled on another (Okun et al., 2019).

Evidence from recent DRN recordings suggests that 5-HT neurons can signal across multiple timescales and different temporal components of 5-HT neuron activity can be tuned to the same stimulus in different ways (Cohen et al., 2015; Cohen & Grossman, 2020; Hayashi et al., 2015). In support of this idea experiments in Chapter 3 demonstrated that single putative 5-HT neurons (i.e., slow regular spiking DRN neurons) could signal an aversive foot-shock stimulus across different timescales. Many neurons had elevated spike rates during a block of repeated foot-shock presentations but had decreased spiking activity during a short time period following foot-shock onset.

As neuronal correlations could influence the physiology and signaling of 5-HT neurons, and neuronal correlations and 5-HT signaling can exist at multiple timescales, it is important to identify the timescale on which correlations among DRN neurons occur. Doing so may identify the temporal component of the 5-HT signal that is most influenced by local DRN dynamics.

The timescale on which neuronal correlations occur has been investigated by studies that have quantified the relationship between the strength of pairwise neuronal correlations and duration of binning intervals used when converting spike trains to regularly sampled spike count time series (Bair et al., 2001; Kodama et al., 2018; Smith & Kohn, 2008). Correlations calculated using short durations have been interpreted as spike timing synchrony whereas those when long durations were used have been interpreted as co-fluctuations in spike rate. An increase from low to large correlation magnitude with increasing integration duration has been interpreted as evidence for correlated spike rates but not spike timings.

4.1.5 Chapter aims

The aim of this chapter was to investigate how the dynamics of putative 5-HT neurons were related to neighboring neurons in the DRN network. Silicon probe recordings were performed in the DRN of urethane anesthetized mice to generate simultaneous neuronal recordings. The neurochemical identities of DRN neurons were estimated to be either putative 5-HT, GABA, or generic non-5-HT on the basis of their electrophysiological properties.

The timescales on which DRN neurons were correlated was investigated by quantifying the magnitude of pairwise neuronal spike count correlations as a function of the duration of the bin width used when binning spike trains to regularly sampled spike count timeseries.

To investigate how activity was coordinated among putative 5-HT neurons, the heterogeneity of pairwise spike count correlations between these neurons was quantified, and the extent to which their correlations were organized into modular neuronal ensembles was assessed.

To investigate how the activities of putative 5-HT neurons were related to those of putative GABA and non-5-HT neurons, the distribution of pairwise spike count correlations between putative 5-HT and these neurons was quantified and the relationship between these correlations and ensemble membership was assessed.

Finally, several general features of neuronal interactions within the DRN were quantified, including their stability over time and the relationship between pairwise correlation magnitude and the distance between neurons.

4.2 Methods

4.2.1 Dataset

The work in this chapter further analyzes the silicon probe dataset generated in Chapter 2. See the Methods section of Chapter 2 for a detailed description the methodology used to generate this dataset.

The dataset contains recordings of 566 DRN neurons made from 26 urethane-anesthetized mice. The spontaneous activity of neurons was recorded for 30-mins. In Chapter 2, neurons were classified into the following three neuron-types based on their electrophysiological properties: slow regular spiking with broad waveforms, slow and irregular spiking without broad waveforms, and fast firing neurons with narrow waveforms (SR, SIR and FF neurons, respectively).

4.2.2 Correlation measures

4.2.2.1 Spike count time series

Prior to computing all neuronal correlation measures, spike trains were converted to regularly sampled spike count time series. Unless stated otherwise, the sampling interval (hereafter referred to as bin width) was one second.

4.2.2.2 Pairwise measures

Spike count correlations (r_{sc}) were computed by calculating the Pearson correlation coefficient between two simultaneously recorded neurons. This measure was interpreted as the overall correlation between a neuron pair.

The partial spike count correlation ($r_{sc-partial}$) of a neuron pair was computed by calculating the partial correlation coefficient of their spike count time series adjusting

for all other simultaneously recorded neurons. This measure was interpreted as the remaining correlation between a neuron pair after having adjusted for common coupling to other neurons in the population.

To determine whether pairwise correlation measures (i.e., r_{sc} or $r_{sc-partial}$) were greater than that expected by chance, observed value magnitudes were compared to null distributions. Null values were generated by computing the corresponding measure (either r_{sc} or $r_{sc-partial}$) between surrogate inter-spike-interval shuffled neuron pairs. An initial P value was calculated as the proportion of values in the null distribution that were at least as large as the observed value. Where measures were computed for multiple neuron pairs, initial p values were corrected for multiple comparisons using the Benjamini Hochberg method.

4.2.2.3 *Population coupling*

The population coupling coefficient of a neuron was generated by taking the Pearson correlation coefficient of its spike count time series with the summed spike count time series of all other simultaneously recorded neurons. Thus, this measure correlated a neuron's spiking activity with the population activity in the DRN network.

To determine whether observed population coupling coefficients were greater than what would be expected by chance, observed coupling magnitudes were compared to null distributions. Null values were generated by shuffling the inter-spike-intervals of surrogate neurons prior to computing its Pearson correlation coefficient with the population spike count time series. An initial P value was calculated as the proportion of values in the null distribution that were at least as large as the observed value. Where population coupling was computed for multiple neurons, initial p values were corrected for multiple comparisons using the Benjamini Hochberg method.

4.2.3 Detection of neuronal ensembles

The detection of neuronal ensembles involved the following steps: identifying the number of putative ensembles contained in each recording, testing whether the resultant partition of neuronal correlations was more modular than what would be expected by chance, assigning neurons to putative neuronal ensembles, and, finally, filtering ensembles according to a set of inclusion criteria.

4.2.3.1 Detection of putative ensembles

The number of putative ensembles in each recording was estimated by identifying the number of clusters that resulted in the partition of neuronal correlations that maximized a modularity metric (Newman, 2010). Rectified spike count correlations ($r_{sc-rect}$) were computed by first calculating r_{sc} and then setting negative values to zero. Putative ensembles were identified by clustering the matrix of pairwise $r_{sc-rect}$ using the spectral clustering algorithm. The number of clusters k was varied between two to eight. For each k the clustering algorithm was ran 20 times, each time storing the resultant modularity of the clustering partition. The k and run that maximized modularity was chosen for further analysis. This clustering step resulted in a modularity score and assigned each neuron to a putative ensemble. Spectral clustering was performed using the ‘scikit-learn’ python package and modularity was computed using the ‘NetworkX’ python package (Hagberg et al., 2008; Pedregosa et al., 2012).

4.2.3.2 Modularity test

To determine whether the neuronal correlations from a recording were more modular than that expected by chance, the modularity of the best partition was compared to a null distribution of 100 values generated by computing the partition modularity

generated following shuffling of pairwise $r_{sc-rect}$ values. Observed modularity values were classified as greater than chance if they were greater than 95% of values in the null distribution. This step resulted in each recording being classified as significantly modular or not.

4.2.3.3 Ensemble inclusion criteria

For recordings that were considered significantly modular, a set of criteria was used to exclude or include each putative ensemble. Ensembles were included if they had a mean within-ensemble $r_{sc-rect}$ that was greater than 0.1 and an edge expansion was greater than 95% of null values generated by permuting observed ensemble membership 1000 times. Edge expansion was computed using the ‘NetworkX’ python package.

$$Q = \frac{1}{2m} \sum_{ij} \left(A_{ij} - \gamma \frac{k_i k_j}{2m} \right) \delta(c_i, c_j)$$

Equation 2: Modularity.

Where m is the number of pairwise neuronal correlations; $\mathbf{A}$ is the adjacency matrix of rectified spike count correlations ($r_{sc-rect}$); k_i is the summed $r_{sc-rect}$ of neuron i ; γ is the resolution parameter (set to 1); $\delta(c_i, c_j)$ is one if neuron i and j are members of the same ensemble and zero otherwise (Hagberg et al., 2008; Newman, 2010).

4.2.4 Analysis of the timescale of DRN neuronal correlations

To measure the timescales on which the spiking activity of DRN neurons were correlated, the r_{sc} of all neuron pairs was calculated using different bin widths (0.05, 0.1, 0.5 and 1 secs). These durations were chosen as previous studies performing

similar analyses have reported neuronal correlations occurring on these timescales (Bair et al., 2001; Kodama et al., 2018; Okun et al., 2019; Smith & Kohn, 2008). Neuron pairs were inferred to be correlated to a greater degree at one timescale than another if the magnitude of r_{sc} was higher, and proportion of neuron pairs with non-zero r_{sc} was greater at that timescale with respect to the other. The magnitude of r_{sc} was compared across timescales using a t-test. The proportion of non-zero correlations was compared across timescales using a chi-square contingency test. Statistical tests were performed using the ‘SciPy’ python package (Virtanen et al., 2020).

4.2.5 Analysis of correlations between SR neurons

The extent to which the spike rates of SR neurons were correlated with that of other SR neurons was quantified by calculating the r_{sc} of all simultaneously recorded SR-SR neuron pairs. The extent to which relationships between the spike rates of SR neurons were unique interactions among those neurons, or instead reflected common coupling to population activity, was investigated by quantifying the distribution of $r_{sc\text{-}partial}$ among SR-SR neuron pairs and the population coupling coefficient of SR neurons. To determine whether correlations among SR neurons were organized such that they formed neuronal ensembles, the presence of neuronal ensembles was first determined in a neuron-type agnostic manner, and then the neuron-type composition of resultant ensembles was inspected.

4.2.6 Analysis of correlations between SR neurons and SIR and FF neurons

The relationships between the activities of SR neurons with those of neighboring SIR and FF neurons were investigated by quantifying r_{sc} of all SR-SIR and SR-FF neuron

pairs and determining whether SIR and FF neurons were members of the same ensembles as SR neurons.

The extent to which the neuronal correlations of FF neurons were consistent with them negatively regulating neighboring SR neurons was investigated by two analyses. The first compared the proportion of FF neurons with significantly negative population coupling coefficients to corresponding proportions of SR and SIR neurons. A significant association between neuron-type and negative population coupling was determined using a chi-square contingency test and post hoc proportion z-test. The second analysis compared the proportion of SR-FF interactions occurring between non-ensemble neurons that were negative to corresponding proportions of SR-SR and SR-SIR neurons. A significant association between neuron-type combination and proportion of negative correlations was determined using a chi-square contingency test and post hoc proportion z-test. The ‘SciPy’ python package was used to compute the chi-square contingency test and the ‘statsmodels’ python package was used to compute the proportion z-tests (Seabold et al., 2010; Virtanen et al., 2020).

4.2.7 Quantification of general properties of DRN neuronal correlations

4.2.7.1 Stability

The stability of DRN neuronal correlations over time was investigated by quantifying the similarity of neuronal correlations computed during the first and second halves of the 30-min recording session (i.e., each half = 15 min). For each session half, r_{sc} was computed between all simultaneously recorded neuronal pairs using a 1-sec bin width to convert spike trains to spike count time series. The similarity of neuronal

correlations across session halves was parameterized as the Spearman correlation coefficient of r_{sc} distributions. The similarity of correlation structure was parameterized as the Spearman correlation coefficient of neuronal clustering coefficients. The ‘SciPy’ python package was used to calculate Spearman correlation coefficients and the ‘NetworkX’ python package was used to calculate neuronal clustering coefficients (Hagberg et al., 2008; Virtanen et al., 2020).

4.2.7.2 *Distance*

The relationship between r_{sc} magnitude and distance between neuron recording sites was estimated using a generalized additive model. This modelled the magnitude of the r_{sc} of a neuron pair as a function of the neuron-type combination (SR-SR, SR-SIR and SR-FF), and the estimated distance between its neurons.

The distance between a neuron pair was estimated by first assigning the position of each neuron as the channel on which its spiking had maximal amplitude, and then calculating the Euclidean distance between channel locations. To account for non-linear relationships, distance was modelled using a cubic regression spline with a separate term for each neuron-type combination. The significance of model terms was assessed using F-tests. Model fitting and evaluation was performed using the ‘mgcv’ R package (Pedersen et al., 2018).

4.2.7.3 *Predictive power of neuronal properties for determining correlation strength and ensemble membership*

Whether the correlation strength (r_{sc}) and ensemble membership of a neuron pair could be predicted from either their neuron-type and their distance was quantified using a supervised modelling approach.

Separate models were fit for the prediction of r_{sc} magnitude and common ensemble membership. A ridge regression model was used for r_{sc} prediction, and a decision tree classifier was used for common ensemble membership prediction. Both models modelled distance using spline terms and incorporated interaction terms for neuron-type combinations. Models were trained using a 5-fold cross validation routine and evaluated as the mean out-of-sample performance across folds. The evaluation metric used for ensemble membership prediction was F1 score and that used for r_{sc} prediction was R^2 .

Bootstrap hypothesis tests were used to determine whether the performance of models was greater than that expected by chance. For both r_{sc} and common ensemble membership, a null distribution of evaluation metrics was generated by shuffling these target variables prior to model fitting 1000 times. Observed model performance was classified as significantly better than chance if it was greater than 95% of corresponding null values. Models were fit and evaluated using the ‘scikit-learn’ Python package (Pedregosa et al., 2012).

4.2.8 Software and statistics

Data processing, transformations, and statistical analysis was performed using custom-written software in R, Python, MATLAB, and SQL. All statistical analysis used an alpha level of 0.05 and multiple comparisons were accounted for where appropriate using Benjamini-Hochberg adjustments.

4.3 Results

4.3.1 Dataset

Silicon probe recordings were made in the DRN of 22 urethane anesthetized mice. Recordings were made during 30-min of spontaneous activity. A total of 565 single neurons were recorded (mean count per session: 25.68 ± 2.71). Neurons were categorized into three neuron-types on the basis of their electrophysiological properties (see Methods): slow regular neurons with broad waveforms, slow irregular neurons without broad waveforms and fast firing neurons with narrow waveforms (SR, SIR and FF neurons, respectively). A total of 243 SR, 228 SIR, and 94 FF neurons were recorded (mean count per session: 11.06 ± 1.47 SR, 10.36 ± 1.81 , SIR and 4.7 ± 0.68 FF).

4.3.2 Timescale of DRN neuronal correlations

To investigate how correlations between DRN neuron pairs varied across temporal scales, spike count correlations (r_{sc}) were calculated between all pairs of simultaneously recorded neurons using several bin widths for the conversion of spike trains to spike count time series (see Methods).

The magnitude of r_{sc} was low for small duration bin widths but increased linearly with increasing bin width duration (Figure 4.1). Thus, the mean r_{sc} magnitude when the bin width was 1-sec (0.206 ± 0.002 ; indicative of correlations of spike rate) was significantly higher than the corresponding value when the bin width was 0.05 sec (0.035 ± 0.001 ; indicative of correlations of spike timing; $t = -102.84$, $p < 0.05$). Similarly, there was a monotonic increase in the proportion of neuron pairs that were significantly correlated with increasing bin width size. The proportion of neuron pairs

that had greater than chance r_{sc} was higher when the bin width was 1-sec ($34.6 \pm 0.5\%$) than the corresponding proportion when the bin width was 0.05 sec ($60.9 \pm 0.005\%$; $\chi^2 = 1196.58$, $p < 0.05$).

These results indicated that the spike rates of DRN neuron pairs were correlated with each other to a greater extent than their spike timings.

4.3.3 Correlations between SR neurons

The overall correlation between the spike rates of SR neurons was investigated by quantifying the r_{sc} between all pairs of simultaneously recorded SR-SR neurons (1 sec bin width). Most SR-SR neuron pairs had significantly correlated spike rates ($60.3 \pm 1.2\%$, total pairs = 1724; Figure 4.2). The types of correlations were heterogeneous (Figure 4.2 – A) : some SR-SR neuron pairs had positively correlated spike rates ($47 \pm 1.2\%$), whereas others were either negatively correlated ($13.3 \pm 0.8\%$) or not significantly correlated ($39.7 \pm 1.2\%$). Furthermore, within these correlation classifications there was heterogeneity in the degree of correlation. For example, among positively correlated SR-SR neuron pairs, some were highly correlated whereas others were weakly correlated (Figure 4.2).

To investigate whether the correlations among SR-SR neuron pairs reflected bespoke interactions between neuron pairs or common coupling to higher order dynamics, two further metrics were quantified: the partial spike count correlation ($r_{sc-partial}$) of SR-SR neuron pairs and the population coupling coefficient of SR neurons (see Methods). The $r_{sc-partial}$ of most SR-SR neuron pairs ($84 \pm 0.9\%$) was not greater than chance (Figure 4.2 – B). Conversely, most SR neurons ($78.3 \pm 3.8\%$) had greater than chance population coupling coefficients (Figure 4.2). Among significantly coupled SR neuron

pairs, the predominant valance was positive ($81.2 \pm 3.0\%$), meaning that these neurons had higher spike rates during periods of elevated DRN population activity. These results indicated that correlations among SR-SR neuron pairs did not reflect bespoke interactions between those neurons but rather that most SR neurons were correlated with DRN population activity.

To investigate whether the heterogeneous r_{sc} among SR neurons reflected a modular organization, the presence of a modular correlation structure in each recording session was assessed. Moreover, where modular structures were detected (hereafter referred to as neuronal ensembles), the r_{sc} of SR-SR neuron pairs were compared whether they were members of the same ensemble or not. The structure of neuronal correlations was significantly modular in $77.3 \pm 8.9\%$ of recordings with the mean ensemble size being 11.32 ± 1.1 neurons. The magnitude of r_{sc} of SR-SR neuron pairs in the same ensemble was significantly larger than that of SR-SR pairs that were not in the same ensemble (0.317 ± 0.008 versus 0.1 ± 0.006 ; $t = 14.14$, $p < 0.05$).

Taken together, these results demonstrated that the spike rates of many SR neurons were correlated with neighboring SR neurons and that these correlations were heterogeneous. Most neuronal correlations among SR-SR neuron pairs did not reflect unique interactions between those neurons but instead common coupling to dynamics that involved groups of SR neurons. Specifically, DRN neuronal correlations had a modular structure and SR neurons that were members of the same ensemble were more correlated than those that were not.

4.3.4 Correlations between SR neurons and SIR and FF neurons

The relationships between the spike rates of SR neurons and those of SIR and FF neurons was investigated by calculating r_{sc} for all simultaneously recorded SR-SIR and SR-FF neuron pairs (1 sec bin width). Most SR-SIR neuron pairs were significantly correlated ($60.8 \pm 0.9\%$, total pairs = 2740), with $73.5 \pm 1.1\%$ of these correlated pairs being positively correlated and $26.5 \pm 1.1\%$ being negatively correlated. Similarly, most SR-FF neuron pairs were significantly correlated ($60.3 \pm 1.2\%$, total pairs = 1724), with $77.9 \pm 1.3\%$ of these correlated pairs being positively correlated and $22.1 \pm 1.1\%$ being negatively correlated. For both SR-SIR and SR-FF neuron pairs, the magnitude of correlations was variable among correlation classifications. For example, some positively correlated neuron pairs had only weakly correlated spike rates while others were strongly correlated (Figure 4.3).

Analysis of the neuron-type composition of DRN neuronal ensembles revealed that both SIR and FF neurons were members of neuronal ensembles (Figure 4.3). Most neuronal ensembles contained multiple neuron-types with many containing SR alongside SIR and FF neurons (Figure 4.3). The r_{sc} magnitudes of same-ensemble SR-SIR neuron pairs were significantly larger than corresponding r_{sc} magnitudes among not-same-ensemble SR-SIR neuron pairs (same: 0.24 ± 0.006 , not: 0.15 ± 0.004 ; $t = 12.65$, $p < 0.05$). Similarly, the r_{sc} magnitudes of same-ensemble SR-FF neuron pairs were significantly larger than corresponding r_{sc} magnitudes among not-same-ensemble SR-FF neuron pairs (same: 0.32 ± 0.010 , not: 0.22 ± 0.01 ; $t = 7.76$, $p < 0.05$). These results demonstrated that many DRN neuronal ensembles contained SIR and FF neurons in addition to SR neurons and that the neuronal heterogeneity of SR-SIR and SR-FF r_{sc} values can in part be accounted for by differing r_{sc} magnitudes

depending on whether neurons in the pair were members of the same neuronal ensemble.

To investigate whether the dynamics of (putative GABA) FF neurons were consistent with them tonically inhibiting population activity, the proportion of FF neurons with significantly negative population coupling coefficients was compared with corresponding proportions of SR and SIR neurons. Only a minority of FF neurons (13.6%) had significantly negative population coupling coefficients and there was no association between neuron-type and negative population coupling ($\chi^2 (2) = 0.436$, $p > 0.05$; Figure 4.3).

To investigate whether the dynamics of FF neurons were consistent with them inhibiting SR neurons depending on ensemble membership, the proportion of negative not-same-ensemble SR-FF neuron pair r_{sc} values was quantified and compared to corresponding proportions of SR-SR and SR-SIR neuron pairs. There was a significant association between neuron-type combination and whether not-same-ensemble r_{sc} values were negative ($\chi^2 (2) = 8.48$, $p < 0.05$; Figure 4.3). The proportion of negative not-same-ensemble SR-FF r_{sc} values (27.3%) was significantly higher than corresponding proportions of SR-SR and SR-SIR neuron pairs (19.9% and 23.3%, respectively; $Z = 2.33$, $P < 0.05$). This demonstrated that, compared to other DRN neuron-types, a greater proportion of FF neurons were negatively correlated with neighboring SR neurons with which they were not members of the same ensemble.

4.3.5 Quantification of general properties of DRN correlations

4.3.5.1 Temporal stability of DRN neuronal correlations

To determine whether neuronal correlations of DRN neurons were stable over time, neuronal correlations were calculated independently during the first and second halves of the 30-min recording session and the similarity of resultant correlations was quantified. The Spearman correlation of first and second half r_{sc} distributions was 0.45 ± 0.01 , indicating that the neuron pairs that had high r_{sc} during one session half were likely to also have high r_{sc} during the second session half. The Spearman correlation of $r_{sc-rect}$ clustering across session halves was 0.43 ± 0.04 , indicating that neurons with clustered $r_{sc-rect}$ during the first session were likely to also have clustered $r_{sc-rect}$ during the second session. These results indicated that the structure of DRN neuronal correlations was stable over the 30-min recording session.

4.3.5.2 Relationship of distance between neuron pairs and DRN neuronal correlations

To investigate the relationship of distance between neuron pairs and DRN neuronal correlations, the magnitude of pair's r_{sc} was modelled as a function of the estimated distance between, and neuron-types of, its constituent neurons (see Methods). There was a significant main effect of neuron-type combination ($F(2) = 81.79$, $p < 0.05$). The distance spline terms for SR-SR ($F(8.66) = 5.94$, $p < 0.05$) and SR-SIR ($F(1.23) = 4.41$, $p < 0.05$) neuron pairs were significant. There was no similar effect of distance for SR-FF neuron pairs ($F(8.85) = 1.72$, $p > 0.05$). This indicated that r_{sc} magnitudes differed by the neuron-type combination of the pair and distance. Also, the effect of distance differed by neuron-type combination. For SR-SR neuron pairs, there was an initial linear decrease in r_{sc} magnitudes with increasing distance over 0 - 0.2 mm, after

which point correlations no longer reduced with increasing distance (Figure 4.4). For SR-SIR neuron pairs, there was a linear decrease with increasing distance that did not plateau (Figure 4.4). SR-FF neuron had high r_{sc} magnitudes at both short and long distances (Figure 4.4).

4.3.5.3 *Predictive power of DRN neuronal properties for neuronal correlations*

A predictive modelling approach was used to quantify the extent to which the heterogeneity in the correlation between SR neuron pairs could be accounted for by the neuron-types of the neurons involved and distance between them. Thus, a ridge regression model was trained using a 5-fold cross validation routine to predict the out of sample r_{sc} magnitude of SR-SR, SR-SIR and SR-FF neuron pairs (see Methods). The mean out-of-sample R^2 of this model was 0.036. While low, this was significantly higher than those of identically parametrized models trained on shuffled data ($p < 0.05$; Figure 4.4).

A similar approach was used to quantify the extent to which the common ensemble status of a neuron pair involving a SR neuron (i.e., whether the component neurons of a neuron pair were or were not members of the same ensemble) could be predicted from the distance between those neurons as well as their neuron-types. Thus, out-of-sample common ensemble status predictions were made on SR-SR, SR-SIR, and SR-FF neurons pairs using a decision tree classifier. The mean out-of-sample F1 score was 0.53. This, while low, was significantly higher than predictions of identically parametrized models trained on shuffled data ($p < 0.05$; Figure 4.4).

These results demonstrated that, while the distance between neurons and neuron-types of partner neurons could to some extent predict the correlations of SR neurons,

the correlations of these neurons remained highly variable even when these properties were accounted for.

4.4 Discussion

4.4.1 Timescale of DRN correlations

The present study investigated the timescale on which DRN neuron pairs were correlated by quantifying spike count correlations (r_{sc}) using binning intervals of different durations. This revealed that neuron pairs were mostly uncorrelated when using short-duration intervals but that correlations increased with increasing interval duration. As r_{sc} measures synchrony in spike timing when short duration intervals are used and co-fluctuation of spike rate when long duration intervals are used (Bair et al., 2001; Cohen & Kohn, 2011; Smith & Kohn, 2008), the results indicate that DRN neurons tend to have synchronized spike rates rather than spike timings.

The slow timescale of neuronal correlations observed among DRN neurons in the present study has been identified in other brain regions. For example, using a similar methodology to that used here, it was demonstrated that neurons of the superficial and deep layers of visual cortex had high correlations when long duration intervals were used but low when short duration intervals were used (Smith et al., 2013). As discussed in later sections, the use of long duration intervals revealed that DRN neuronal correlations had a modular structure. Similar timescale-dependent modular correlation structures have been reported in studies quantifying neuronal correlations in other brain regions. For example, it was reported that neuronal correlations of isolated somatosensory cortex had a modular structure when long duration bin

widths were used to compute correlations but were sparsely correlated when low duration bin widths were used (Kodama et al., 2018).

DRN neuronal correlations occurring at the level of spike rate but not spike timing could have several implications for how the activity of 5-HT neurons are influenced by neighboring neurons within the nucleus. Firstly, because DRN neurons including 5-HT neurons can signal using both phasic changes in spike rate and tonic changes in spike timing (Cohen et al., 2015; Cohen & Grossman, 2020; Hayashi et al., 2015), and because correlated activity among neurons can affect signal propagation from those neurons (Carillo-Reid & Yuste, 2020; Carrillo-Reid et al., 2019; Humphries, 2018), the slow timescale signaling from DRN neurons including 5-HT neurons may be selectively influenced by the activity of neighboring neurons. Secondly, because 5-HT neurons are proposed to be negatively regulated by neighboring 5-HT and non-5-HT neurons within the DRN (Boothman et al., 2006; Sharp et al., 2007), and because inhibitory relationships can present at the level of neuronal correlations (Adler et al., 2013; Cardin, 2018; Diba et al., 2014), the paucity of neuronal correlations of spike timing and presence of correlations of spike rate indicates that local regulatory influences may ultimately affect the spike rate and not the precise spike timings of 5-HT neurons.

Two aspects of the methodology used in the present study may have influenced these results. Firstly, recordings were made under urethane anesthesia. Measurements of neuronal correlations from the same cortical neurons during anesthesia and wakefulness indicated that correlations were inflated during anesthesia with respect to wakefulness (Ecker et al., 2014). Therefore, it is possible that the correlations of DRN spike rates observed in the present study are larger than those that occur in other

settings. The extent to which anesthesia influenced the strength of slow timescale correlations of DRN neurons could be clarified in subsequent work by measuring DRN neuronal correlations in different brain states such as during wakefulness or NREM sleep.

Secondly, the correlation measurements were based on recordings of spontaneous activity. While there is evidence that DRN neurons including 5-HT neurons signal on both fast and slow timescales when certain physiological stimuli are presented (Cohen et al., 2015; Cohen & Grossman, 2020; Hayashi et al., 2015), it is not known whether these neurons do so during spontaneous activity. For this reason, the absence of fast-timescale correlations observed in the present study could reflect these relationships being state-dependent, i.e., absent during spontaneous activity and present during fast-timescale signaling of the 5-HT system. Future studies could clarify this issue by measuring DRN neuronal correlations during a setting wherein DRN neurons would be expected to signal on multiple timescales such as during a behavioral task during which aversive stimuli are presented continually over multiple trials (Cohen et al., 2015; Hayashi et al., 2015).

4.4.2 Correlations between SR neurons

The coordination of activity between (putative 5-HT) SR neurons was investigated by quantifying the correlation between SR-SR neuron pairs and determining whether SR neurons were members of neuronal ensembles. This revealed that many SR neuron pairs had correlated activity but that relationships were heterogeneous. The correlations of DRN neurons had a modular structure with many SR neurons being members of neuronal ensembles that, on average, contained over 10 neurons. These results indicate that 5-HT neurons have heterogeneous interactions with neighboring

5-HT neurons, being strongly correlated with those with which they form neuronal ensembles and less strongly with non-ensemble neurons.

The heterogeneous nature of correlations among 5-HT neurons observed here is in accord with correlations reported in other neuronal populations (de la Rocha et al., 2007; Smith et al., 2013; Smith & Kohn, 2008).

That SR neuron pairs had heterogeneous correlations may be related to the recently discovered variation in properties of subsets of 5-HT neurons (Abrams et al., 2004; Huang et al., 2019; Okaty et al., 2019; Ren et al., 2019; Weissbourd et al., 2014). For example, subsets of 5-HT neurons were found to have distinct patterns of afferent input and varying levels of 5-HT receptor expression (Abrams et al., 2004; Okaty et al., 2019). Thus, the heterogeneous correlations among SR neurons could arise from this neuronal population comprising multiple subclasses of 5-HT neurons with diverse anatomical and molecular properties.

There are similarities between the modular dynamics of SR neurons identified in the present study and that of noradrenergic neurons in the LC (Shahryar Noei et al., 2022; Totah et al., 2018, 2019). Following the identification of LC neuronal ensembles, subsequent work demonstrated that ensemble neurons had similar neuroanatomical and functional properties with respect to non-ensemble neurons (Shahryar Noei et al., 2022; Totah et al., 2018), supporting hypotheses of LC neurons using a differentiated coding scheme (Totah et al., 2019). The DRN 5-HT system has also been hypothesized to operate in a differentiated manner (Okaty et al., 2019; Ren et al., 2018). Whether ensembles in the DRN represent functional modules of the 5-HT system could be explored further by determining whether 5-HT neurons in neuronal

ensembles have common physiological properties such as tuning to behavioral variables and anatomical connectivity.

4.4.3 Correlations between SR neurons and SIR and FF neurons

The relationships between SR neurons and SIR and FF neurons (estimated to be 5-HT, generic non-5-HT, and GABA neurons, respectively) were investigated by quantifying the correlation between these neuron-types and investigating how these correlations were related to ensemble membership. Both SIR and FF neurons had heterogeneous correlations with SR neurons and were often found to be members of SR-neuron containing neuronal ensembles. Additionally, it was found that, relative to other neuron type combinations, SR-FF neuron pairs were often negatively correlated when not members of the same ensemble. These findings suggest that 5-HT neurons have heterogeneous interactions with neighboring non-5-HT neurons, and that the latter neurons may be members of the same neuronal ensembles as 5-HT neurons.

The correlations of SR neurons with SIR and FF neurons may result from common afferent input from external brain regions. In support of this idea, cell type specific mapping of input onto DRN 5-HT and GABA neurons revealed several brain regions including the prefrontal cortex and hypothalamus that project onto both these neuron types (Weissbourd et al., 2014). Diversity of neuronal correlations could arise from input differentially targeting subpopulations of neurons. For example, input from the PFC was found to synapse differentially on subpopulations of 5-HT and GABA neurons depending on their localization within the DRN (Weissbourd et al., 2014). This link between localization within the DRN and afferent connectivity has also been reported by other studies (Abrams et al., 2004; Peyron et al., 1998) and is consistent with the observation from the present investigation that correlation strength was

related to distance. Both SR-SR and SR-SIR neuron pairs located closer together had higher magnitude correlations than those further apart.

That there was no relationship between correlation strength and distance among SR-FF neuron pairs may be related to the extensive anatomical connections of DRN GABA neurons. Axonal reconstruction of DRN fast spiking GABA neurons revealed that these neurons had long-distance local projections throughout the nucleus (Allers & Sharp, 2003).

Should the coordination of activity between 5-HT and non-5-HT neurons depend on external input, the relationships between these neurons may change when the pattern of input changes. This is because input from several brain regions projects differentially onto DRN neuron-types. For example, neurons of the prefrontal cortex projects onto 5-HT neurons to a greater degree than GABA neurons (Weissbourd et al., 2014). State-dependence could be assessed by future work by quantifying neuronal correlations between 5-HT and non-5-HT neurons in multiple brain states such as during wakefulness, NREM sleep or as an animal engages in a behavioral task.

The role of non-5-HT neurons in DRN ensembles is not clear. One possibility is that these neurons are projection neurons. Indeed, non-5-HT DRN neurons project out of the DRN, can be tuned to behavioral variables, and modulations of their activity can alter behaviors. For example, glutamatergic DRN neurons project to the VTA are tuned to reward-related behaviors, and optogenetic activation of VGLUT3-expressing neurons (a marker for glutamate neurons) in the DRN modulated reward-related behaviors (Z. Liu et al., 2014; Qi et al., 2014). An alternative possibility is that these neurons coordinate ensemble activity. Consistent with this hypothesis, a greater proportion of FF neurons than SR and SIR neurons were negatively correlated with

SR neurons with which they were not members of the same ensemble. These FF neurons may not be projection neurons but instead local GABA neurons that orchestrate intra-DRN dynamics, indirectly promoting the activity of one ensemble by suppressing the activity of other 5-HT neurons.

The role of non-5-HT neurons in an ensemble could be further explored by determining whether they are more or less likely than 5-HT neurons in an ensemble to project to external brain regions. Such an experiment could be performed by detecting neuronal ensembles using *in vivo* electrophysiology and estimating whether neurons project out of the DRN using antidromic electrical/optogenetic stimulation approaches.

4.4.4 Conclusion

The present study provides a detailed description of neuronal interactions between electrophysiologically-defined neuron types (SR, putative 5-HT; SIR, putative non-5-HT; FF, putative GABA) within the DRN. It was demonstrated that correlations between neuron pairs were timescale dependent, with the spike timing of neurons being weakly correlated and spike rates more strongly correlated. The results suggest that 5-HT neuron pairs have heterogeneous correlations with subsets of 5-HT neurons forming neuronal ensembles, consistent with theories of a differentiated 5-HT system. Correlations between putative 5-HT and non-5-HT neurons were quantified, revealing complex relationships, and indicating that non-5-HT neurons may contribute in some way to 5-HT neuronal ensembles.

4.5 Figures and tables

4.5.1 Results figures

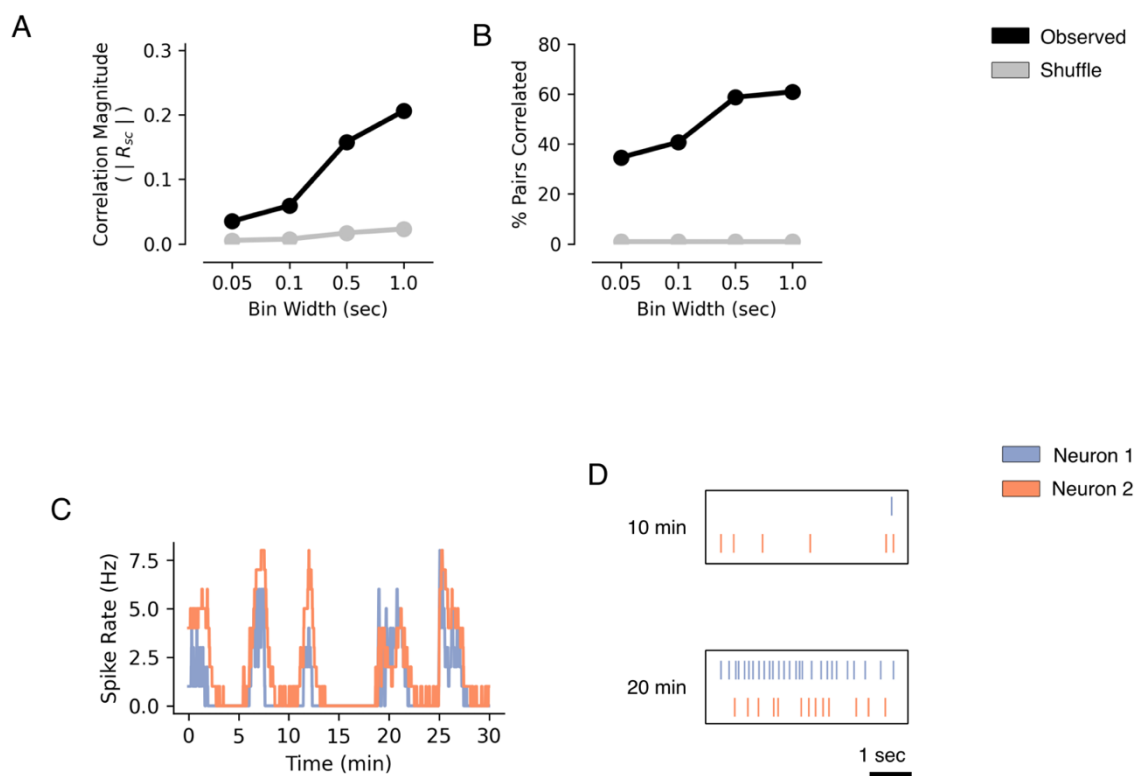


Figure 4.1: Timescale of DRN neuron correlations.

- A)** Spike count correlation magnitude of neuron pairs as a function of the bin width used to convert spike trains into time series of regularly sampled spike counts. Error bars cover bootstrapped 95% confidence intervals.
- B)** Percentage of neuron pairs that had significantly non-zero correlation as a function of the bin width used to convert spike trains into time series of regularly sampled spike counts. Data in black correspond to the observed data while data in grey correspond to correlations of inter-spike interval shuffled surrogates. Error bars cover bootstrapped 95% confidence intervals
- C)** Spike rate over time of two example neurons that were classified as being positively correlated with each other. The spike rates of both neurons fluctuated over time and were correlated across neurons.

- D)** Two raster plots showing 5 secs of spiking activity at different time points from the two example neurons plotted in **C**. The spike times of these neurons were not synchronized across neurons.

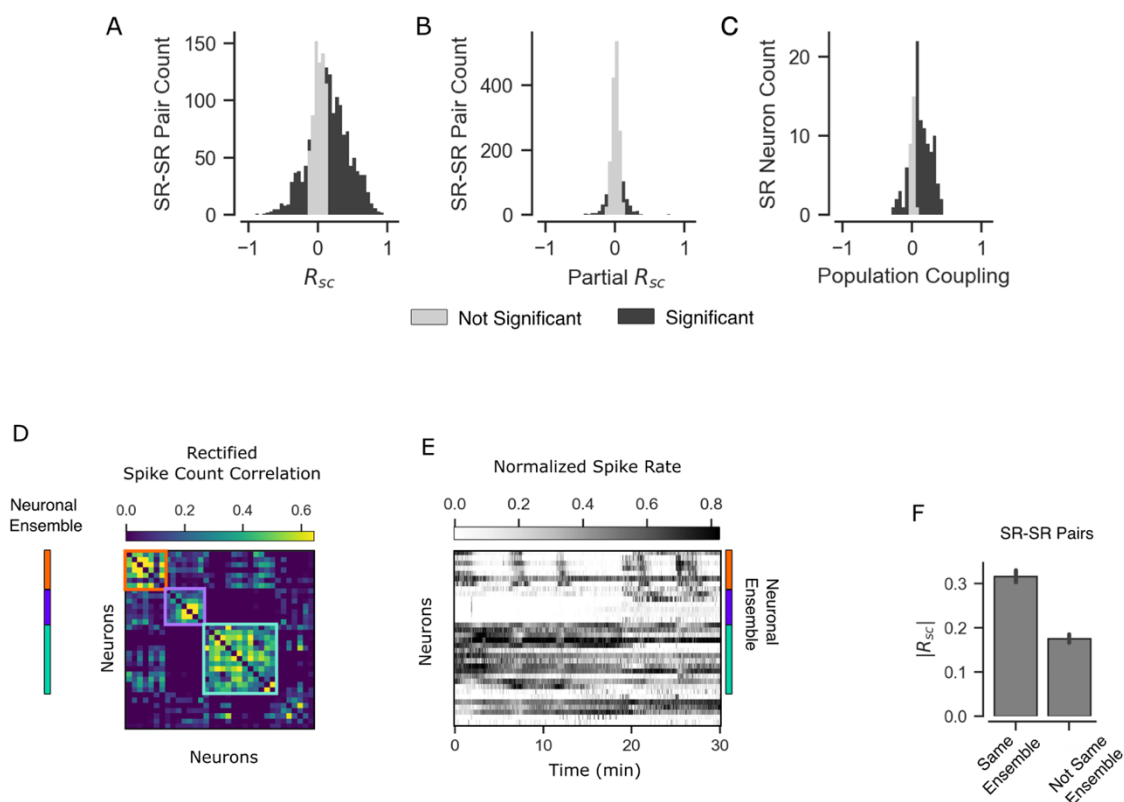


Figure 4.2: Correlations between SR neurons.

- A)** Distribution of spike count correlations among pairs of simultaneously recorded SR neurons (1 sec bin width). Histogram bins are colored according to whether neuron pairs had significantly non-zero correlation (see color key below).
- B)** Distribution of partial spike count correlations among pairs of simultaneously recorded SR neurons (1 sec bin width). Histogram bins are colored according to whether neuron pairs had significantly non-zero partial correlation (see color key below).
- C)** Distribution of population coupling coefficients (correlation between one neuron and population activity) of SR neurons. Histogram bins are colored according to whether neurons had significantly non-zero correlation (see color key below).
- D)** Example of a rectified spike count correlation matrix that contained three neuronal ensembles (see left color key and box around neurons).

- E)** Spike rate (0-1 normalized) of neurons from the recording session shown in **D**. Neurons are ordered by ensemble membership (see right color key, same y-axis ordering as **D**).
- F)** Magnitude of spike count correlation among pairs of SR neurons that either were or were not in the same ensemble. Error bars cover bootstrapped 95% confidence intervals.

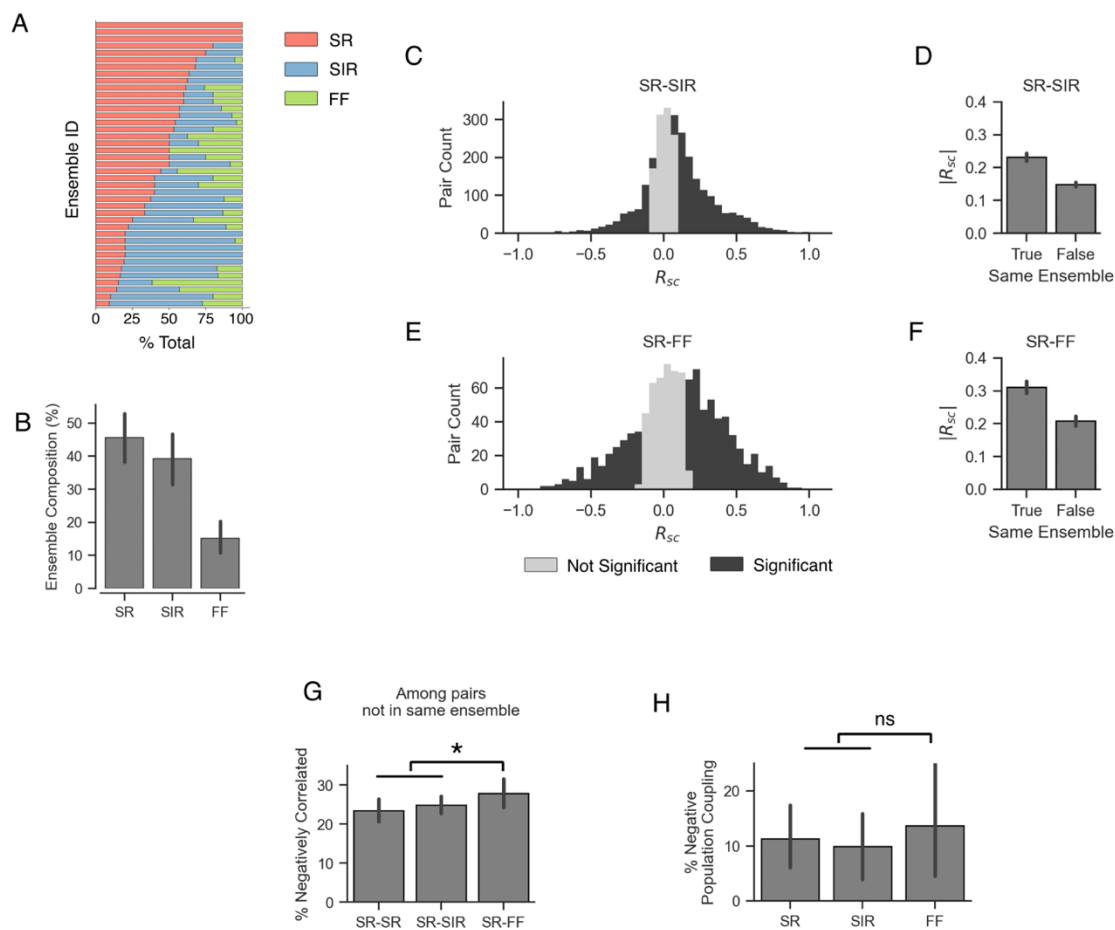


Figure 4.3: Correlations between SR neurons and SR and FF neurons.

- A)** Neuron-type composition (see right color key) of each ensemble (rows).
- B)** Mean neuron-type composition across all ensembles. Error bars cover bootstrapped 95% confidence intervals.
- C)** Distribution of spike count correlations among pairs of simultaneously recorded SR and SIR neurons (one sec bin width). Histogram bins are colored according to whether neuron pairs had significantly non-zero correlation (see color key below).
- D)** Magnitude of spike count correlation among pairs of SR and SIR neurons that either were or were not in the same ensemble. Error bars cover bootstrapped 95% confidence intervals.

- E)** Distribution of spike count correlations among pairs of simultaneously recorded SR and FF neurons (one sec bin width). Histogram bins are colored according to whether neuron pairs had significantly non-zero correlation (see color key below).
- F)** Magnitude of spike count correlation among pairs of SR and FF neurons that either were or were not in the same ensemble. Error bars cover bootstrapped 95% confidence intervals.
- G)** Proportion of non-same-ensemble correlations that were significantly negative by neuron-type combination. Error bars cover bootstrapped 95% confidence intervals. A significant post-hoc proportion Z-test is denoted by `\*`.
- H)** Proportion of neurons with negative population coupling coefficients by neuron-type. Error bars cover bootstrapped 95% confidence intervals. A not significant post-hoc proportion Z-test is denoted by `ns`.

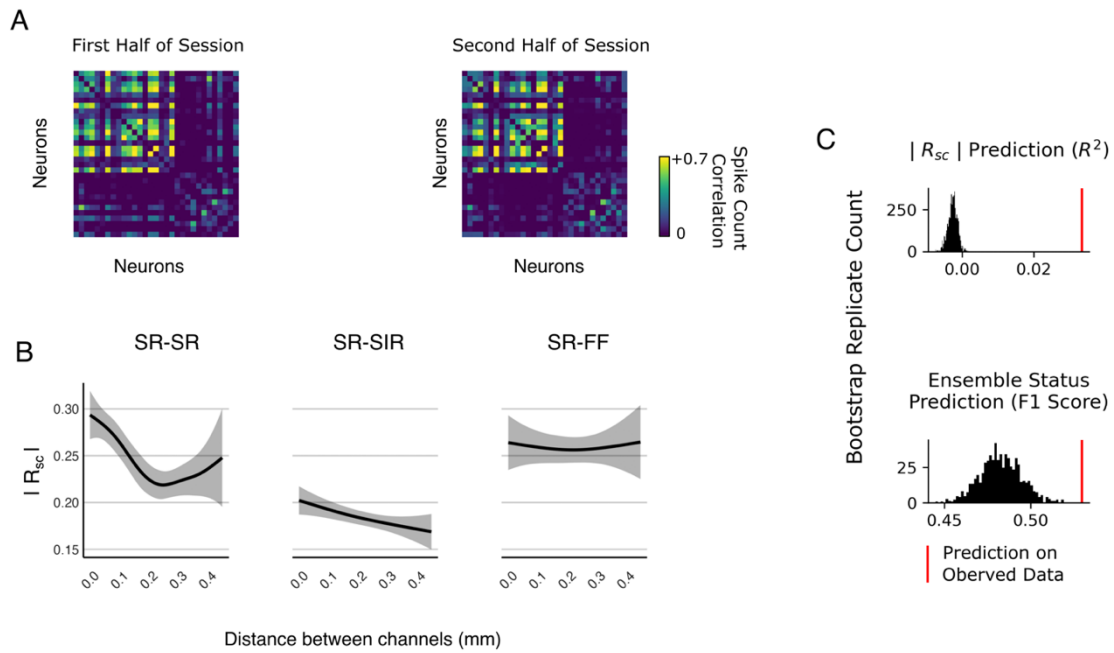


Figure 4.4: Properties of DRN correlations.

- A)** Rectified spike count correlation matrices of an example session calculated using data from the first and last 15 mins of the recording session (left and right, respectively).
- B)** Spike count correlation magnitude as a function of the distance between the recorded neurons by neuron-type combination (column facets). Bands cover 95% confidence intervals around the mean.
- C)** Performance of two prediction models (red lines) relative to shuffle controls (black histogram bins). The top plots the performance of a model predicting a neuron pair's spike count correlation given the neuron-types and distance between neurons. The bottom plots the performance of a model predicting whether a neuron pair were a member of the same ensemble given their neuron-types and the distance between them.

Chapter 5: Electrophysiological analysis of the DRN 5-HT system following SSRI treatment

5.1 Introduction

5.1.1 Selective serotonin reuptake inhibitors and the DRN 5-HT system

Selective serotonin reuptake inhibitors (SSRIs) are the first line treatment for depression and anxiety disorders (Pilling et al., 2011). The primary pharmacological action of these drugs is inhibition of the 5-HT transporter (DeVane & Cl, 1999; Hyttel & Hyttel, 1994). While SSRIs have been proven effective at addressing some of depression's major symptoms they are not effective in all patients (Cipriani et al., 2018; D. Warden et al., 2007) and often produce side effects like sexual dysfunction and emotional blunting (G. Gartlehner et al., 2011; Marazziti et al., 2019; Shelton & Tomarken, 2001).

There has been a large research effort to uncover the effects of SSRIs on 5-HT neurons but the ways by which they alter several aspects of 5-HT neurophysiology remains poorly understood. In particular, it is unclear whether SSRIs have differential effects on the diverse subclasses of 5-HT neurons that have recently been uncovered (Huang et al., 2019; Okaty et al., 2019; Ren et al., 2019). Additionally, it is not known whether SSRIs effect the interactions occurring between 5-HT neurons and other neurons in the DRN. Finally, although it is known that SSRIs alter the spontaneous firing of 5-

HT neurons (Blier & de Montigny, 1994; Blier & El Mansari, 2013; Sharp et al., 2007), it is not known whether they alter their sensitivity to physiological inputs such as aversive stimuli (Cohen et al., 2015; Hayashi et al., 2015; Schweimer & Ungless, 2010) the processing of which is altered in patients on SSRI treatment (Marazziti et al., 2019; McCabe et al., 2010).

5.1.2 Potential diverse effects of SSRIs on 5-HT and non-5-HT DRN neurons

A number of previous *in vivo* electrophysiological studies have investigated the effects of SSRIs on the firing of 5-HT neurons in rodents (i.e., slow, regular firing neurons with broad waveforms) (Blier & de Montigny, 1994; Blier & El Mansari, 2013). It is well known that acute SSRI administration causes an immediate inhibition of 5-HT neuron firing, and that this effect often attenuates during continued treatment (Blier & de Montigny, 1983; Chaput, Blier, et al., 1986; Czachura & Rasmussen, 2000; Mansari et al., 2005). The current thinking is that SSRIs increase extracellular 5-HT in the DRN which activates somatodendritic 5-HT_{1A} autoreceptors (Bel & Artigas, 1992; Blier & de Montigny, 1994; Blier & El Mansari, 2013; Gartside et al., 1995; Invernizzi et al., 1994) which desensitize with continued SSRI treatment (Chaput, Blier, et al., 1986; Czachura & Rasmussen, 2000).

Recent single cell RNA sequencing studies have reported the presence of subpopulations of 5-HT neurons with different expression levels of 5-HT_{1A} autoreceptors (as well as other 5-HT receptor subtypes including 5-HT_{5B} and 5-HT₇) (Huang et al., 2019; Ren et al., 2019). These data are in accord with *in vitro* evidence that not all 5-HT neurons are inhibited by bath application of 5-HT_{1A} receptor agonists (Calizo et al., 2011; Kirby et al., 2003; Marinelli et al., 2004). These data raise the

possibility that subpopulations of 5-HT neurons may be differentially sensitive to SSRI treatment.

There is also evidence that SSRI treatment may affect the activity of 5-HT neurons indirectly through actions on local non-5-HT neurons in the DRN which express 5-HT receptors (Sharp et al., 2007). For example, there is evidence that DRN GABA neurons express both 5-HT_{2A} and 5-HT_{2C} receptors and are activated by 5-HT₂ receptor agonist administration to then cause GABA-mediated changes in 5-HT neuron activity (Boothman & Sharp, 2005; R. Liu et al., 2000). Other recent studies have identified expression of 5-HT receptors including 5-HT_{2C} receptors on DRN glutamate and dopamine neurons (Huang et al., 2019). As SSRIs increase extracellular 5-HT in the DRN (Bel & Artigas, 1992; Invernizzi et al., 1994), findings such as these indicate that SSRI treatment may additionally influence the activity of non-5-HT neurons which may in turn impact on the activity of 5-HT neurons.

5.1.3 SSRI actions on DRN network dynamics

Following on from the above, SSRIs could impact the dynamics of the DRN network through interactions with both 5-HT and non-5-HT neurons such as GABA neurons. Previous experiments in this thesis (Chapter 4) identified heterogeneous correlations between putative 5-HT neurons in the DRN: subsets of neuron pairs were highly correlated and formed neuronal ensembles, while other neuron pairs were weakly correlated. Diverse correlations were also identified between putative 5-HT and non-5-HT neurons: these neurons could be members of the same neuronal ensembles but, especially for pairs of putative 5-HT and GABA neurons, were often negatively correlated when they were not. A recent study utilizing calcium imaging and graph theoretic analysis methods in zebrafish provided evidence that SSRIs altered network

dynamics (Burgstaller et al., 2019). Specifically, fluoxetine altered the structure of neuronal interactions as indexed by the clustering of neuronal correlations.

There are several mechanisms through which SSRIs might impact on neuronal correlations in the DRN. For example, SSRI treatment might alter interactions between 5-HT neuron pairs by the desensitization of 5-HT_{1A} autoreceptors. Altered network dynamics during SSRI treatment could also result from elevated levels of neuroplasticity, which has been linked to changes in neuronal correlations and evidence suggests may be sensitive to concentrations of extracellular 5-HT (Alejandre-García et al., 2022; Lesch & Waider, 2012).

5.1.4 Effect of SSRIs on aversive processing

As noted above, it is well known that SSRIs inhibit the spontaneous firing of 5-HT neurons (Chaput, Blier, et al., 1986; Czachura & Rasmussen, 2000), but it is not known whether they affect the sensitivity of these neurons to physiological stimuli. As outlined in Chapter 1, the firing of 5-HT and other neurons in the DRN are sensitive to an aversive stimuli such as foot-shocks (Cohen et al., 2015; Hayashi et al., 2015; Schweimer & Ungless, 2010). Chapter 3 performed a detailed analysis of the effects of a foot-shock stimulus on neuronal firing in the DRN network. It was found that both putative 5-HT and non-5-HT neurons had diverse responses to the stimulus occurring over multiple timescales.

SSRI treatment is associated with the blunting of aversive processing (Marazziti et al., 2019; McCabe et al., 2010), and previous fMRI studies have associated this effect with altered neural processing in the amygdala (McCabe et al., 2010). It is not known whether SSRIs additionally alter the sensitivity of 5-HT neurons to aversive stimuli.

5.1.5 Chapter aims.

The present study used silicon probe recordings to address three gaps in knowledge regarding the effects of SSRIs on the DRN 5-HT system. First, experiments examined the effects of SSRI treatment on the spontaneous firing properties of putative 5-HT and non-5-HT neurons with analyses directed towards determining (1) whether SSRIs had diverse effects across putative 5-HT neurons and (2) whether SSRIs altered the firing of putative non-5-HT neurons. Next, experiments examined whether SSRI treatment altered neuronal correlations between putative 5-HT neurons and between putative 5-HT and non-5-HT neurons. Finally, experiments examined the effect of SSRI treatment on the responsivity of DRN neurons to an aversive foot-shock stimulus. Citalopram was chosen as an example SSRI because of its high selectivity for the 5-HT transporter compared to other drugs in this class (Hyttel & Hyttel, 1994).

5.2 Methods

5.2.1 Citalopram treatment

Adult male C57BL6/J wild type mice (n = 12) were group housed and maintained on a 12-hour light/dark cycle, with food and water provided ad libitum. All procedures were conducted in compliance with the Animal Scientific Procedures Act 1986 (ASPA, UK) and the guidelines of the University of Oxford.

Mice were randomly allocated to either treatment with continued citalopram (10 mg/kg; CIT, n = 7) or saline vehicle (SAL, n = 5). Intraperitoneal injections were administered twice daily for 14 days (injections given at 7 am and 7 pm) before the electrophysiological recordings under urethane anesthesia on the 15th day.

5.2.2 Silicon probe recordings

5.2.2.1 *Recording protocol*

Recordings contained the following blocks occurring in this order one after another:

1. **Spontaneous activity** (30 mins): Spontaneous activity was recorded.
2. **Foot-shock** (10 mins): Foot-shocks were delivered in the same way as outlined in Chapter 3. See later section for further details.
3. **Pre-drug** (10 mins): Spontaneous activity was recorded.
4. **Citalopram challenge** (20 mins): An acute citalopram challenge (10 mg/kg citalopram hydrobromide in sterile saline; Abcam; intraperitoneal injection) was administered at the start of the block and then spontaneous activity was monitored for a further 20 mins.

5.2.2.2 *Implantation and data processing*

This study employed the same silicon probe recording methodology outlined in Chapter 2. See that chapter for details.

5.2.2.3 *Classification of neuron types*

Neurons were classified into three putative neuron types — slow regular firing (SR), slow irregular firing (SIR), and fast firing (FF) neurons — based on their mean spike rate, spike regularity, and waveform width during a 30-min period of spontaneous activity.

Electrophysiological properties were calculated in the same way as outlined in Chapter 2. Briefly, the mean spike rate was calculated as the number of spikes per second during the 30-min block. Spike regularity was defined as the CV2 of inter-

spike-intervals and waveform width was defined as the time duration between time spike initialization and repolarization of average waveforms. Average waveforms were constructed by averaging raw traces around the 1000 first spike times (see Chapter 2 for details).

Classification used the K-Means model from Chapter 2 that was used in that Chapter to cluster neurons from treatment-naïve animals into the three neuron types. Neurons from the current Chapter were assigned to the neuron type whose centroid they were closest to in Euclidean distance. This classification was implemented using the 'scikit-learn' Python package (Pedregosa et al., 2012).

5.2.3 Analysis of firing properties

A statistical analysis was conducted to determine whether the relative frequency of neuron types differed between SAL and CIT mice. For this purpose, a chi-square contingency test was employed to ascertain if there was an association between group and neuron type. For post hoc tests, Benjamini-Hochberg adjusted proportion z-tests compared the relative frequency of each neuron type by group. The chi-square test and proportion z-tests were fit using the 'SciPy' and 'statsmodels' Python packages, respectively (Seabold et al., 2010; Virtanen et al., 2020).

Differences between the electrophysiological properties of neurons of SAL and CIT mice were assessed using linear models. A separate model was fit for each electrophysiological property: spike rate, spike regularity, and waveform width. Each model was parameterized as follows: neuron type (whether the neuron was SR, SIR, or FF), group (whether the neuron was recorded in a mouse that was a member of the SAL or CIT group), and group- neuron type interaction. Main effects were assessed

using F tests. In cases where the group or group-neuron type interaction was significant, Tukey adjusted post hoc t-tests were used to compare estimated marginal means. Model fitting was performed using the 'stats' R package, and post hoc tests were computed using the 'emmeans' R package (Lenth, 2022).

5.2.4 Analysis of the effects of an acute citalopram challenge

Random slopes and intercepts models were employed to quantify the effects of the citalopram challenge on neuronal activity. A separate model was fit for each neuron type, with identical parameterization. The dependent variable was the spike counts of a neuron during a 30 sec bin. The fixed effects were block (pre-drug or citalopram challenge), group, and group-block interaction. The random effects were random block slope and random intercepts for neuron ID, accounting for the heterogeneity of spike rates and block effects across neurons. The significance of fixed effects was assessed using F tests, implemented in the 'LmerTest' R package (Kuznetsova et al., 2017). In cases of significant block or group-block interaction effects, estimated marginal means were compared using Tukey-adjusted post hoc tests implemented in the 'emmeans' R package (Lenth, 2022).

5.2.5 Analysis of neuronal interactions

5.2.5.1 Correlation measures and detection of neuronal ensembles

Spike count correlations (r_{sc}), partial spike count correlation ($r_{sc-partial}$) and neuronal population coupling coefficients were calculated as outlined in Chapter 4. Similarly, neuronal ensembles were detected in the same way as outlined in Chapter 4.

5.2.5.2 *Analysis of the timescale, structure, and temporal stability of neuronal correlations*

Timescale: r_{sc} was computed between all simultaneously recorded neuron pairs having used 0.05, 0.1, 0.5, and 1 sec bin widths to convert spike trains into spike count time series. A linear mixed-effects model was employed to determine the effect of bin width and group on r_{sc} magnitude. The fixed effects were bin width, group, and group-bin width interaction. The random effects were random intercepts for neuron pair ID and neuron type. F tests and Tukey-adjusted post hoc tests were used to determine the significance of effects and neuron pairs were interpreted to be correlated to a greater extent at one timescale with respect to another if they had higher magnitude r_{sc} when using the bin widths of that timescale.

Structure: The effect of SSRI treatment on the organization of neuronal correlations was assessed by determining whether the presence and properties of neuronal ensembles differed between SAL and CIT mice. Ensembles were detected independently for each recording. Differences in the proportion of recordings that had neuronal ensembles were determined using a chi-square contingency test. Differences in the number of ensembles per recording and the mean within-ensemble rectified r_{sc} ($r_{sc-rect}$) was determined using Mann-Whitney U tests implemented in the ‘SciPy’ Python package (Virtanen et al., 2020).

Stability: r_{sc} was calculated independently during the first and second halves of the 30-min spontaneous activity block. For each recording, the similarity between r_{sc} first and second halves was quantified using spearman correlation coefficients. A significant difference between the stability of SAL and CIT recordings was assessed

using a Mann-Whitney U test comparing corresponding distributions of spearman correlation coefficients.

5.2.5.3 Analysis of negative interactions between DRN neuron pairs

It was hypothesized that citalopram treatment may reduce negative interactions between 5-HT neurons through desensitization of 5-HT_{1A} autoreceptors. It was reasoned that attenuated negative interactions would present as reductions in the prevalence of both negative r_{sc} and significant $r_{sc-partial}$ relationships between neuron pairs.

Two identically parameterized logistic regression models were employed to evaluate the association between group and the proportion of neuron pairs having (1) negative r_{sc} and (2) significant $r_{sc-partial}$ relationships. The main effects were group, neuron type combination (e.g., SR-SR, SR-SIR, etc.), distance, and group-neuron type interaction. Distance between neuron pairs was estimated in the same way as outlined in Chapter 4. Likelihood ratio tests were used to assess the significance of main effects, and adjusted proportion z-tests conducted with the 'emmeans' R package compared estimated marginal proportions for post hoc tests (Lenth, 2022).

5.2.5.4 Analysis of the neuron type composition of neuronal ensembles

Two analyses were conducted to assess whether continued SSRI treatment effected the diversity of neuron types in DRN neuronal ensembles. First, a proportion Z-test was used to compare across groups the proportion of SR-neuron containing neuronal ensembles that also contained at least one SIR or FF neuron. Second, a Mann-Whitney U-test was used to compare the neuron type entropy of CIT and SAL neuronal ensembles.

5.2.5.5 *Analysis of the non-ensemble correlations of SR neurons*

A logistic regression model was employed to assess the effect of continued citalopram treatment on the interactions between SR neurons and other neurons that were not members of the same ensemble (not-same-ensemble neuron pairs). The dependent variable was whether a neuron pair had significantly negative r_{sc} . The independent variables were group, same ensemble (whether the neuron pair were members of a common ensemble), neuron type combination (SR-SR, SR-SIR, or SR-FF) and corresponding interaction terms. Likelihood ratio tests assessed the significance of fixed effects, and proportion z-tests were used for post-hoc analysis.

5.2.6 **Analysis of the effects of an aversive foot-shock stimulus**

5.2.6.1 *Hardware and experimental protocol*

As noted above, this Chapter employs the same foot-shock paradigm that was detailed in Chapter 3. Briefly, neuronal activity was monitored during two experimental blocks: pre-shock (10 mins; last 10 mins of the spontaneous activity block) and foot-shock (10 mins). During the pre-shock block, spontaneous activity was monitored. During the foot-shock block, mild electric foot-shocks were delivered to the hind paw of mice every 2 seconds (2 msec pulse duration, 2 mA). Shocks were controlled using an Arduino and delivered using a A360 stimulus isolator (World Precision Instruments).

5.2.6.2 *Analysis of foot-shock response profiles*

The slow timescale response of each neuron was determined using t tests to compare spike rates in pre-shock and foot-shock blocks (30 sec bin width), resulting in each

neuron being classified as activated, inhibited, or not responsive. T tests were adjusted for multiple comparisons using the Benjamini-Hochberg method.

Similarly, fast timescale responses were determined using t tests to compare PSTH spike counts in two post-shock windows to those occurring at other latencies (50 – 200 msec, and 500 – 800 msec; 10 msec bin width). These windows were chosen based on observed DRN 5-HT system modulations in Chapter 3 (50 – 200 msec) and exploratory PSTH analysis in CIT animals (500 – 800 msec). Based on the results of the t tests, each neuron's response was categorized as activated, inhibited, or not responsive. T tests were adjusted for multiple comparisons using the Benjamini-Hochberg method.

For each neuron type, chi-square contingency tests examined associations between groups (SAL, CIT) and response profiles (inhibited, activated, not responsive) for each set of response profiles (slow timescale, fast timescale 50 – 200 msec, fast timescale 500 – 800 msec). Where significant associations were observed, post-hoc proportion Z-tests compared response-type frequency by group.

5.2.6.3 Analysis of population coding

An analysis was performed to determine whether continued citalopram treatment was associated with a change in the putative population coding of the foot-shock stimulus. Decoders predicted foot-shock status (pre or post shock onset) based on DRN neuronal activity. Thus, decoders of the 50 – 200 msec window predicted whether neuronal activity occurred during that window or during a 200 – 0 msec pre shock window.

The class of decoding models used were light gradient-boosting machines. Decoders were trained using 5-fold stratified sampling cross-validation. Performance was evaluated by averaging F1 scores across held-out folds. This analysis used the 'LightGBM' Python package for decoders and the 'scikit-learn' package for model training and evaluation.

The following classes of decoders were trained and evaluated:

- **Single neuron decoders:** Activity from a single neuron.
- **Single neuron shuffle decoders:** Activity from one neuron with foot-shock correlation removed by shuffling inter-spike-intervals before alignment.
- **Population decoders:** Activity from all neurons in a recording session.
- **Population shuffle decoders:** Activity from all neurons in a session with foot-shock correlation removed, using the single neuron shuffle approach.
- **Limit decoders:** Activity from a random subset of neurons in a session (e.g., 5-neuron limit decoder sampled 5 random neurons without replacement).

Decoders were compared across groups using a linear mixed effects model with random intercepts. The dependent variable was evaluated F1 score, the fixed effects were group, decoder class (single neuron or population decoder), and group decoder class interaction. The random effects were recording session. F-tests were used to determine the significance of fixed effects.

Decoders were compared within groups using paired t-tests. For example, the performance of single neuron decoders in SAL mice were compared to corresponding single neuron shuffle decoders from the same session.

5.2.7 Software and statistics

Data processing, transformations, and statistical analysis was performed using custom-written software in R, Python, MATLAB, and SQL. All statistical analysis used an alpha level of 0.05 and multiple comparisons were accounted for where appropriate using Tukey and Benjamini-Hochberg adjustments.

5.3 Results

5.3.1 Silicon probe recordings of DRN neurons

Silicon probe recordings were made in the DRN of mice on either continued citalopram treatment (CIT, $n = 7$) or continued saline treatment (SAL, $n = 5$). A total of 431 (35.92 ± 6.74 neurons/mouse) DRN neurons were recorded, 273 neurons recorded in the CIT group (39 ± 9.52 neurons/mouse) and 158 neurons in the SAL group (31.6 ± 8.85 neurons/mouse). The number of SR, SIR and FF neurons recorded in the SAL and CIT groups is shown in Table 5.1. The total number of simultaneously recorded neuron pairs of each combination of neuron types is shown in Table 5.2.

5.3.2 Effect of repeated citalopram on DRN neurons

A first set of analyses investigated the electrophysiological properties of DRN neurons recorded in SAL and CIT groups. A chi-square analysis found that the relative frequency (i.e., prevalence) of neuron types differed by group ($\chi^2(2) = 12.77$, $p < 0.05$; Figure 5.1). Post hoc tests revealed that there was no significant difference in the relative frequency of SR neurons between groups (SAL = 52.2%, CIT = 43.9%, $Z = -1.66$, $p > 0.05$). However, the relative frequency of FF neurons was lower (SAL = 13.5%; CIT = 6.3%, $Z = -2.53$, $p < 0.05$) and that of SIR neurons was higher (SAL = 34.2%; CIT = 49.8%, $Z = 3.12$, $p < 0.05$) in the CIT versus SAL group.

Linear models were then employed to investigate potential groupwise differences in electrophysiological properties. This analysis revealed that the spike rate of SR and FF neurons recorded in the CIT group was lower than those recorded in the SAL group (Figure 5.1). Thus, there was a significant group-neuron type interaction ($F(2) = 3.069$, $p < 0.05$), and significant difference in mean firing rate across groups for SR neurons (SAL = 3.69 Hz, CIT = 2.33 Hz; t ratio = 3.95, $p < 0.05$) and FF neurons (SAL = 14.74 Hz, CIT = 12.11 Hz; t ratio = 3.49, $p < 0.05$).

Interestingly, waveform analysis found that the waveform width of SIR neurons was broader in the CIT versus SAL group. Thus, there was a significant group-neuron type interaction ($F(2) = 8.35$, $p < 0.05$) with post hoc t -tests revealing significant differences (SAL = 1.3 msec, CIT = 1.64 msec; t ratio = -3.78; $p < 0.05$). Further inspection of waveforms of SIR neurons identified a subpopulation having broad waveforms on the scale typically associated with SR neurons (Figure 5.1).

Overall, these results demonstrated that SR neurons had lower firing rates in the CIT versus SAL group. Additionally, in the CIT group a population of SIR neurons were identified as having waveforms typically associated with SR neurons. Finally, it was found that the prevalence of FF neurons was lower in the CIT group than the SAL group and those FF neurons that were observed in CIT animals had lower firing rates.

5.3.3 Effect of acute citalopram on DRN neurons

Next, the sensitivity of DRN neurons to an acute citalopram challenge was assessed. In both SAL and CIT mice, neurons were recorded for 10 mins (pre-drug block) before administration of citalopram (10 mg/kg, i.p.). Neurons were then recorded for an additional 20 mins (post-drug block). The effect of acute citalopram administration

on the firing of each neuron type was then assessed using linear mixed effects models (see Methods).

This analysis indicated that SR neurons recorded in both SAL and CIT mice were generally inhibited by citalopram administration with SR neurons in the CIT group being inhibited to a lesser extent than SR neurons in the SAL group (Figure 5.2). Thus, there was a significant fixed effect of group-block interaction ($F(1) = 22.4$, $p < 0.05$), and significant differences of firing rate across blocks for both groups (SAL: t ratio = -8.1 , $p < 0.05$; CIT t ratio = -2.5 , $p < 0.05$), with reductions being significantly attenuated in the CIT versus SAL group (Difference = 1.27 Hz, t ratio = -4.73 , $p < 0.05$).

Analysis of FF neurons indicated that they too were inhibited by acute citalopram but that there was no difference between the CIT and SAL groups (Figure 5.2). Thus, there was a significant fixed effect of block ($F(1) = 5.54$, $p < 0.05$), but no significant group-block interaction ($F(1) = 0.006$, $p > 0.05$). Finally, there was no significant effect of acute citalopram on the firing of SIR neurons in either the CIT or SAL groups (Figure 5.2).

Taken together, these results demonstrated that an acute citalopram challenge inhibited SR neurons to a greater extent in SAL versus CIT mice, but there was no association between group and sensitivity to citalopram among SIR and FF neurons.

5.3.4 Effect of repeated citalopram on DRN neuron correlations

5.3.4.1 Timescale, structure, and stability of neuronal correlations

A series of analyses investigated the effect of continued citalopram treatment on general features of neuronal correlations within the DRN network, specifically their

timescale, stability over time, and organization (as indexed by the presence of neuronal ensembles) (Figure 5.3).

A linear mixed effects model was employed to quantify the relationship between spike count correlation (r_{sc}) magnitude, bin width and group (see Methods). This analysis indicated that for both SAL and CIT mice, neuronal correlations were higher in magnitude on slow timescales than fast timescales, and that on slow timescales r_{sc} magnitudes were lower in CIT with respect to SAL mice (Figure 5.3). Thus, there were significant main effects of bin width (1 sec or 0.01 sec: $F(1) = 9374$, $p < 0.05$), group ($F(1) = 58$, $p < 0.05$), and group-bin width interaction ($F(1) = 76.4$, $p < 0.05$), with post hoc tests revealing significantly higher r_{sc} magnitudes at bin widths of 1 sec than 0.01 sec for both groups (SAL: t ratio = 63.4, $p < 0.05$, CIT: t ratio = 79.4, $p < 0.05$) and significantly higher magnitudes in SAL neuron pairs than CIT at 1 sec bin width (t ratio = 11.241, $p < 0.05$).

Next, the effect of citalopram on the organization of neuronal correlations was determined. There was no association between treatment and the presence of neuronal ensembles as indexed by the proportion of recordings with at least one ensemble (SAL: 80%, CIT: 71.4%, $\chi^2 = 0$, $p > 0.05$; Figure 5.3). Additionally, there was no significant effect of treatment on the number of ensembles per recording (SAL: 3, CIT: 1.5; $U = 4.5$, $p > 0.05$) or the rectified r_{sc} ($r_{sc-rect}$) between ensemble neurons (SAL = 0.25, CIT = 0.24; $U = 54$, $p > 0.05$).

A final analysis investigated whether citalopram treatment altered the stability of neuronal correlations over time, as indexed by the Spearman correlation coefficient of r_{sc} distributions across recording halves (i.e., first, and last 15 mins). There was no

significant difference between the Spearman correlation coefficients of CIT and SAL groups (SAL: 0.38, CIT: 0.49; $U = 7$, $p > 0.05$; Figure 5.3).

Taken together, these results demonstrated that repeated citalopram did not affect general aspects of neuronal correlations between DRN neurons.

5.3.4.2 *Effect of repeated citalopram on negative interactions between DRN neuron pairs*

It was hypothesized that continued citalopram treatment may reduce negative interactions between 5-HT neuron pairs through desensitization of 5-HT_{1A} autoreceptors. Two analyses utilized logistic regression models to investigate the effect of citalopram treatment on the proportion of DRN neuron pairs having (1) significantly negative r_{sc} relationships and (2) significant partial spike count correlation $r_{sc-partial}$ relationships. (Figure 5.4) (see Methods).

This analysis revealed that the proportion of both SR-SR and SR-SIR neuron pairs that had negative r_{sc} relationships was lower in CIT versus SAL mice. Thus, there was a significant main effect of group ($\chi^2 (1) = 9.16$, $p < 0.05$) with post hoc analysis revealing significant groupwise differences in proportions for SR-SR (SAL: 23.17%; CIT: 19.06%; Odds Ratio = 1.28; $p < 0.05$; Figure 5.4) and SR-SIR (SAL: 23.19%; CIT: 19.44%; Odds Ratio: 1.25; $p < 0.05$) neuron pairs. There were no significant differences between neuron pairs of other combinations (Table 5.3).

Citalopram treatment also reduced the proportion of $r_{sc-partial}$ relationships among both SR-SR and SR-SIR neuron pairs. Thus, there was a significant main effect of group ($\chi^2 (1) = 34.07$, $p < 0.05$) and significant post hoc proportion tests for SR-SR (SAL: 13.77%; CIT: 7.19%; Odds Ratio = 2.06; $p < 0.05$; Figure 5.4) and SR-SIR (SAL:

13.47%; CIT: 9.85%; Odds Ratio: 1.43; $p < 0.05$) neuron pairs. Post hoc analysis for pairs of all neuron type combinations is shown in Table 5.4.

These results demonstrated citalopram treatment reduced the proportion of negative r_{sc} and $r_{sc-partial}$ relationships between SR-SR and SR-SIR neuron pairs.

5.3.4.3 *Effect of repeated citalopram on the neuron type diversity of neuronal ensembles*

As neuronal ensembles with heterogeneous neuron types were identified in Chapter 4, an analysis sought to determine whether citalopram treatment was associated with alterations in the neuron type composition of neuronal ensembles. It was found that all ensembles in both groups that contained SR neurons also contained at least one non-SR neuron (i.e., an SIR or FF neuron; Figure 5.5). Moreover, there was no significant difference between groups in the entropy of neuron types within neuronal ensembles (SAL = 1.09, CIT = 1.13, $U = 46.5$, $p > 0.05$).

5.3.4.4 *Effect of repeated citalopram on the SR neuron correlations with non-ensemble SIR and FF neurons*

As it was found in Chapter 4 that SR-FF neuron pairs that were not members of the same ensemble (not-same-ensemble neuron pairs) were often negatively correlated, an analysis investigated whether citalopram treatment altered the relationships between SR-FF not-same-ensemble neuron pairs (Figure 5.5). A greater proportion of SR-FF not-same-ensemble neuron pairs had significantly negative r_{sc} relationships than other neuron type combinations in both SAL (SR-FF: 45%, (SR-SR & SR-SIR): 35%; $Z = -3.1$ $p < 0.05$) and CIT (SR-FF: 47%, (SR-SR & SR-SIR): 29%; $Z = -4.3$, $p < 0.05$) groups with no significant difference in this proportion across groups (Z ratio = -0.34, $p > 0.05$).

5.3.5 Effect of citalopram on the responses of DRN neuron to foot shocks

5.3.5.1 Effect of citalopram on response of single neurons

The effect of repeated citalopram on the response of single neurons to an aversive foot-shock was investigated. Neuronal activity was monitored during two blocks, a pre-drug block (10 mins) during which spontaneous activity was recorded and a foot-shock block (10 mins) during which foot shocks were delivered every 2 seconds (see Methods).

A first analysis investigated the effect of citalopram on the ‘slow timescale’ response profiles of DRN neurons, defined as changes in firing rate occurring between the pre-shock and post-shock block. Thus, the response profile of each neuron was classified as being shock-activated, shock-inhibited or non-responsive and then an association between group and response profile was assessed using a chi-square contingency test. Citalopram treatment did not alter the slow timescale responses of neurons to foot-shock (Figure 5.6): there was no significant association between group and response profile for SR neurons ($\chi^2 (2) = 0.48, p > 0.05$), nor SIR neurons ($\chi^2 (2) = 1.0, p > 0.05$), nor FF neurons ($\chi^2 (2) = 0.86, p > 0.05$).

A subsequent analysis assessed the effect of citalopram on the ‘fast timescale’ response profiles of DRN neurons timescale response of each DRN neurons, defined as altered firing activity in a 50 – 200 msec post-shock window. Citalopram treatment did not alter the fast timescale responses of DRN neurons to the shock stimulus (Figure 5.7): there was no significant association between group and response type for SR neurons ($\chi^2 (2) = 0.48, p > 0.05$), nor SIR neurons ($\chi^2 (2) = 1.0, p > 0.05$), nor FF neurons ($\chi^2 (2) = 0.86, p > 0.05$).

Inspection of PSTHs indicated that unlike mice in the SAL group, mice in the CIT group responded to foot shock during a 500 – 800ms post shock window (Figure 5.7). Indeed, when response profiles were classified using data from this window, there was an association between group and response profile for both SR ($\chi^2 (2) = 17.1, p < 0.05$) and SIR neurons ($\chi^2 (2) = 21.3, p < 0.05$). There was no similar association for FF neurons, possibly due to the small number of these neurons recorded in CIT mice.

During the 500 – 800 msec window a greater proportion of SR neurons in the CIT group were shock-activated (SAL = 30.35%, CIT = 65.78%, $Z = -4.02, p < 0.05$), and a smaller proportion were non-responsive (SAL = 67.86%, CIT = 31.58%, $Z = 4.12, p < 0.05$) compared to SR neurons recorded in SAL mice. A similar observation was made regarding SIR neurons (proportion shock-activated: SAL = 30.35%, CIT = 65.78%, $Z = -4.02, p < 0.05$; proportion non-responsive: SAL = 67.86%, CIT = 31.58%, $Z = 4.12, p < 0.05$).

Taken together, these results demonstrated that in mice treated with citalopram SR neurons showed an enhanced excitatory response to foot-shock, specifically during the 500 – 800 msec post shock window. SIR neurons were similarly affected.

5.3.5.2 Effects of citalopram on population decoding of foot-shocks

In Chapter 3, it was found that the performance of decoders of foot-shock status improved as a function of increasing number of DRN neurons used to make predictions, consistent with neurons employing a population code. An analysis was conducted to investigate whether citalopram treatment altered the relationship between decoding performance and number of DRN neurons used to make predictions. To this end, stimulus decoders were trained to predict whether DRN

neuronal activity occurred during a pre-shock (200 – 0 msec pre-shock) or post-shock (50 – 200 msec post-shock) window (see Methods).

Decoders trained using neurons from CIT and SAL groups had similar performance: decoding performance increased with the number of neurons used by the decoder to predict foot-shock status. Thus, while single neuron decoders had higher performance than shuffle controls (SAL: 0.47; shuffle: 0.417 - $t = 5.97$, $p < 0.05$; CIT: 0.45; shuffle: 0.42, $t = 5.58$, $p < 0.05$), they had lower performance than population decoders (main effect of decoder class: $F(1) = 563.0$, $p < 0.05$;

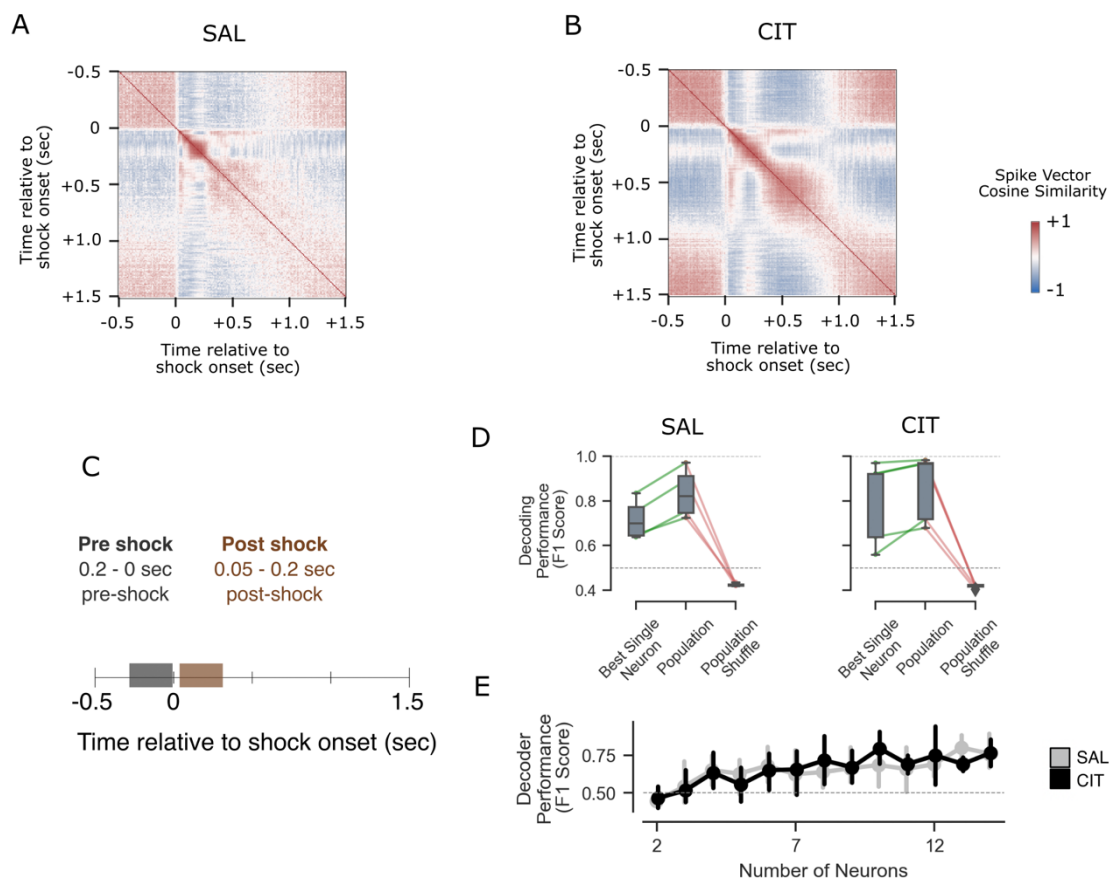


Figure 5.8) for both SAL (Pop: 0.84, Single: 0.42, $t = 15.4$, $p < 0.05$) and CIT (Pop: 0.86, Single: 0.43, $t = 18.3$, $p < 0.05$) mice, with no difference between the size of this

effect by group (group decoder class interaction: $F(1) = 0.5$, $p > 0.05$). Indeed, analysis of the performance of decoders using increasing numbers of randomly sampled neurons demonstrated improved performance with increasing neuron number for both SAL and CIT mice (

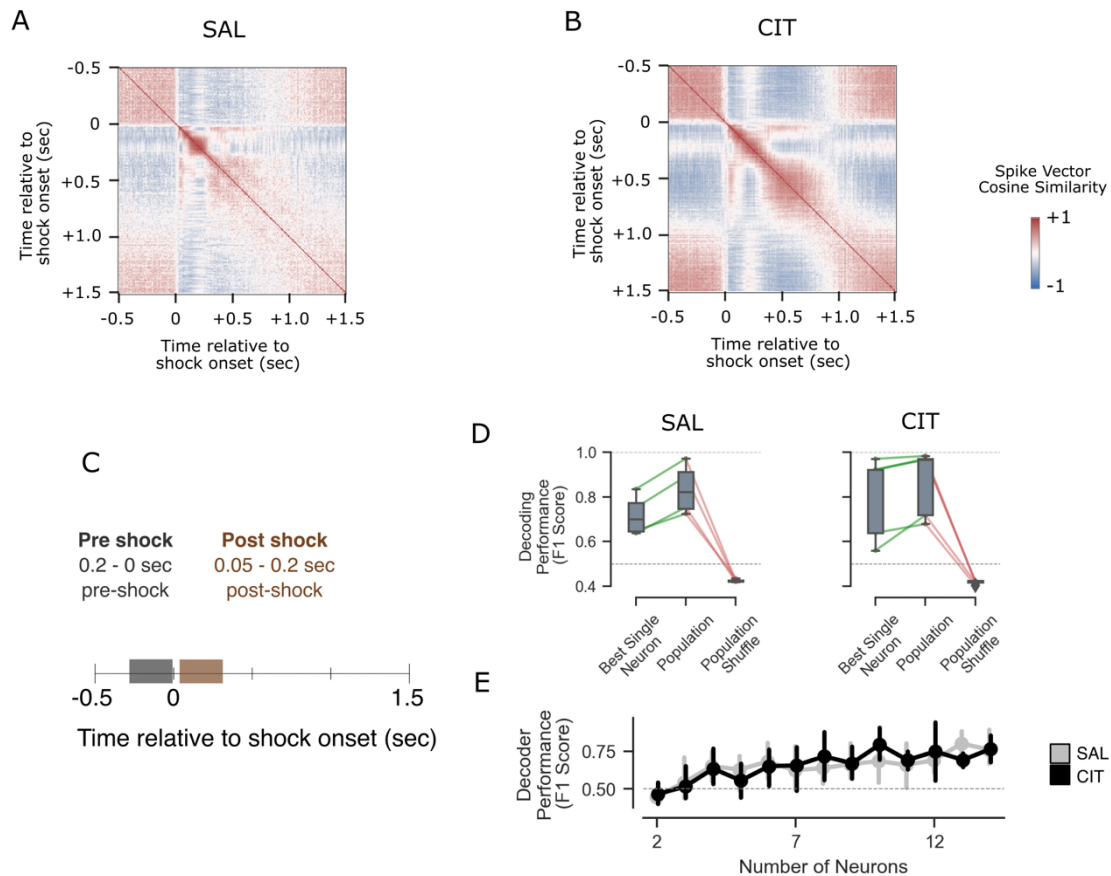


Figure 5.8).

These results indicated that citalopram treatment did not impact the relationship between foot shock decoder performance and number of DRN neurons used to make predictions.

5.4 Discussion

The present study utilized silicon probes to assess the effects of continued treatment with the SSRI citalopram on the firing properties of putative 5-HT and non-5-HT DRN neurons and their sensitivity to an aversive foot-shock stimulus. Continued citalopram treatment: (1) reduced the firing rate of putative 5-HT SR neurons and attenuated their sensitivity to an acute citalopram challenge, consistent with partial 5-HT_{1A} autoreceptor desensitization, (2) generated a subpopulation of broad waveform slow irregular firing neurons that may be a population of 5-HT neurons effected by citalopram in different ways than SR neurons, (3) reduced the negative correlations between the activity of SR-SR neuron pairs, consistent with citalopram attenuating negative interactions between 5-HT neurons, (4) enhanced the excitability of DRN neurons to the foot shock stimulus and (5) reduced the firing rate and prevalence of putative GABA FF neurons. These results provide evidence for SSRIs having diverse effects on subpopulations of 5-HT neurons, indicate that SSRI treatment may alter interactions in the DRN network particularly between 5-HT neurons, suggest that SSRIs may impact non-5-HT neurons in the DRN network, particularly GABA neurons and may alter the dynamics of 5-HT neurons by modulating their sensitivity to physiological stimuli.

5.4.1 Effects of citalopram on spontaneous putative 5-HT neuron firing

In the present study a population of neurons, SR neurons, was identified whose electrophysiological properties (slow and regular firing with broad waveforms) were consistent with both (1) identified 5-HT neurons reported in treatment naive rats and (2) putative 5-HT neurons reported in mice and rats on continued SSRI treatment

(Allers & Sharp, 2003; Chaput, Blier, et al., 1986; Chaput, de Montigny, et al., 1986; Chaput et al., 1991; Czachura & Rasmussen, 2000; Mansari et al., 2005; Schweimer et al., 2011; Schweimer & Ungless, 2010). As reported in previous studies (Chaput, Blier, et al., 1986; Chaput, de Montigny, et al., 1986; Chaput et al., 1991; Czachura & Rasmussen, 2000; Mansari et al., 2005), these putative 5-HT neurons had a lower baseline firing rate and were less inhibited by an acute citalopram challenge compared to SR neurons in saline-treated controls. These findings are consistent with SR neurons in citalopram-treated mice being a population of mostly 5-HT neurons subject to an ongoing inhibitory 5-HT tone mediated by partially desensitized 5-HT_{1A} autoreceptors.

A second population of less regular slow firing neurons with broad waveforms were also noted in citalopram-treated mice. It is plausible that in addition to SR neurons, these neurons were also mostly 5-HT neurons and that the difference in firing patterns between the two subpopulations resulted from their different sensitivity to citalopram treatment. One possibility is that these irregular firing neurons had different expression levels of 5-HT_{1A} autoreceptors than SR neurons and therefore responded in a different way to their desensitization. In support of this idea, transcriptomic studies have identified clusters of DRN 5-HT neurons with different expression levels of 5-HT_{1A} autoreceptors (Huang et al., 2019; Ren et al., 2019) and genetic loss of 5-HT_{1A} autoreceptors has been reported to result in reduced regularity of 5-HT neuron firing (Richardson-Jones et al., 2011).

If SR and broad waveform irregular firing neurons are indeed both 5-HT subpopulations, it would indicate that different 5-HT neurons respond in different ways to SSRI treatment. Interestingly there is evidence that subpopulations of 5-HT

neurons are involved in different behavioral outputs (Marcinkiewicz et al., 2016; Niederkofler et al., 2016; Okaty et al., 2019; Ren et al., 2018). Therefore, it is possible that SSRI treatment effects a specific subpopulation of 5-HT neurons with specific functional properties.

5.4.2 Effects of citalopram on correlations between SR neurons

Analysis of pairwise neuronal correlations revealed two important findings: continued citalopram was associated with a reduction in the prevalence of negative spike count correlations (r_{sc}) and significant partial spike count correlations ($r_{sc-partial}$) between pairs of SR-SR neurons.

One possible explanation for these results is that citalopram attenuated negative interactions between 5-HT neurons. DRN 5-HT neurons are densely interconnected (Weissbourd et al., 2014) and are generally thought to inhibit each other through somatodendritic 5-HT_{1A} autoreceptors (Adell et al., 2002; Sharp et al., 2007). Indeed, 5-HT neurons have commonly been reported to be inhibited by local applications of both 5-HT and 5-HT_{1A} receptor agonists to the DRN (Sharp et al., 2007; Vandermaelen & Aghajanian, 1983). As noted above, continued treatment with SSRIs including citalopram is reported to desensitize 5-HT_{1A} autoreceptors (Chaput, Blier, et al., 1986; Chaput, de Montigny, et al., 1986; Mansari et al., 2005; Raymond, 1991). Therefore, through desensitization of 5-HT_{1A} autoreceptors, SSRI treatment may attenuate inhibitory interactions between 5-HT neurons in the DRN.

It is not clear how altered interactions between 5-HT neurons would impact the functioning of the 5-HT system. One possibility is that attenuated negative correlations reflects reduced sensitivity of 5-HT neurons to feedback control from

neighboring 5-HT neurons in the DRN network. Feedback control has been proposed to be an important function served by the connectivity between 5-HT neurons in the DRN (Sharp et al., 2007). A reduction in the sensitivity of 5-HT neurons to this feedback control during SSRI treatment could result in these neurons having sustained periods of elevated activity.

5.4.3 Effect of citalopram on the sensitivity of SR neurons to foot shocks

The present study measured the effect of citalopram on the sensitivity of SR neurons to an aversive foot-shock stimulus. It was found that neurons in saline and citalopram treated animals had similar responses to the foot-shock stimulus when calculated in the ways identified in Chapter 3 (i.e., changes in firing occurring 50 – 200 msec post shock onset or throughout the 10 min foot shock block). However, an additional period of excitation was noted in citalopram treated animals. Specifically, many SR neurons had elevated firing activity during a 500 – 800 msec post shock window whereas the firing of SR neurons in saline controls was generally not modulated during this window. This finding indicates that the effects of SSRIs on 5-HT neurons extend beyond changes in baseline firing (Blier et al., 1994; Blier & El Mansari, 2013) to altering how these neurons respond to an aversive stimulus.

The underlying mechanism of this increased sensitivity to an aversive stimulus is uncertain. One possibility is that it is related to attenuated feedback control. Loss of 5-HT neuron feedback control could result from 5-HT_{1A} desensitization as noted above. Alternatively, it could arise from the potential tonic inhibition of local GABA neurons, which have also been implicated in feedback regulation of DRN 5-HT neurons (Sharp et al., 2007).

Another possibility is that the enhanced excitations reflect an action of citalopram on the external pathways that drive and regulate 5-HT neurons during aversive processing. In this regard, DRN inputs from both the PFC and LHb are known to influence the firing of 5-HT neurons and have been linked to regulating 5-HT neuron activity during aversive processing (Dolzani et al., 2016; Maier, 2015). The LHb in particular has been proposed to drive excitatory activity in DRN 5-HT neurons during aversive processing (Dolzani et al., 2016). Of note, electrical stimulation of the LHb has been reported to evoke changes in 5-HT neuron activity with a latency comparable to that of the delayed excitation to the foot shock observed in the present study (Varga et al., 2001). However, we are not aware of studies that have examined the effect of SSRI treatment on these inputs to the DRN.

Future studies could investigate some of these potential mechanisms by performing similar measurements to the current study while either administering a selective 5-HT_{1A} receptor antagonist to mimic 5-HT_{1A} autoreceptor function or manipulating PFC/LHb input or DRN GABA neurons using optogenetic strategies. These experiments would likely be feasible as the perturbations noted above have been applied in several recent studies of DRN neurons during aversive processing (Challis et al., 2013; Dolzani et al., 2016; Rozeske et al., 2011; M. R. Warden et al., 2012).

The increase in excitability of putative 5-HT neurons to foot shock observed in the present study may have relevance to studies that have reported deficits in aversive processing in patients on SSRI treatment (Marazziti et al., 2019; McCabe et al., 2010). Previous neuroimaging studies have linked these deficits to altered neuronal processing in the amygdala (McCabe et al., 2010). It is possible that such alterations are a consequence of increased 5-HT release in the amygdala from inputs from DRN

5-HT neurons. Consistent with this idea, several studies in humans have reported a negative correlation between 5-HT release and aversive processing (Nakamura, 2013). For example, tryptophan depletion, which reduces 5-HT release, has been reported to enhance responses to aversive stimuli (Cools et al., 2008; Nakamura, 2013; Roiser et al., 2008).

5.4.4 Effects of citalopram on putative non-5-HT neurons

The current study measured the effects of continued citalopram treatment on the firing of putative non-5-HT neurons. Evidence was found that citalopram treatment inhibited the firing of putative GABA FF neurons. These neurons were less prevalent in CIT mice than SAL controls and those that were observed had lower firing rates. The neurobiological pathways responsible for this effect are unclear. Although both *in vitro* and *in vivo* studies have demonstrated that DRN GABA neurons are sensitive to changes in extracellular 5-HT such as that which occurs in the DRN during SSRI treatment (Bel & Artigas, 1992), most reported effects have been excitatory. Thus, GABA neurons have been reported to have increased activity following the application of 5-HT itself and 5-HT_{2A/C} receptor agonists (Boothman et al., 2006; Boothman & Sharp, 2005; R. Liu et al., 2000).

Interestingly several recent studies have implicated DRN GABA neurons in the acquisition and drug treatment of depression. For example, one recent *in vitro* study found that joint treatment of SSRIs and the atypical antipsychotic olanzapine, which evidence suggests can have enhanced therapeutic benefit versus SSRI monotherapy in some patients (Citrome, 2011; Tohen et al., 2003), found that olanzapine inhibited DRN GABA neurons through blockade of 5-HT₆ receptors (Asaoka et al., 2015). Another study identified a negative correlation between the activity of DRN GABA

neurons and anti-depressant-like behavioral outcomes during repeated social defeat in mice (Challis et al., 2013). This latter study additionally demonstrated that the acquisition of depressive behaviors from social defeat could be prevented by optogenetic inhibition of DRN GABA neurons (Challis et al., 2013).

As noted above, a population of broad waveform slow firing neurons with an irregular firing pattern was identified in citalopram treated mice that could plausibly be 5-HT neurons. Interestingly, several of the effects of citalopram on SR neurons were also noted in SIR neurons. For example, as was the case for SR-SR neuron pairs, the prevalence of SR-SIR neuron pairs with negative r_{sc} and significant $r_{sc-partial}$ relationships was significantly lower in citalopram treated mice than saline controls. Similarly, SIR neurons in citalopram treated mice also had enhanced excitations to foot shocks during the delayed 500 – 800 msec post shock window.

5.4.5 Effects of citalopram on population dynamics

The present study measured the effects of repeated citalopram treatment on two higher order features of DRN population dynamics: the organization of neuronal correlations into neuronal ensembles and the potential population coding of a foot shock stimulus, as indexed by the relationship between decoding performance and number of neurons used by decoders. It was found that neuronal ensembles were present in both saline and citalopram treated animals with there being no association between treatment and either the prevalence or neuron type composition of ensembles. Similarly, foot shock decoders of neurons both of citalopram and saline treated mice had improved performance with increasing neuron number, consistent with these neurons encoding the stimulus using a population code (Laubach et al., 2000; Nicolelis & Lebedev, 2009). These results indicate that these higher order

features of DRN population dynamics can be relatively unchanged by SSRI treatment, despite the marked effects of these drugs on single DRN neurons.

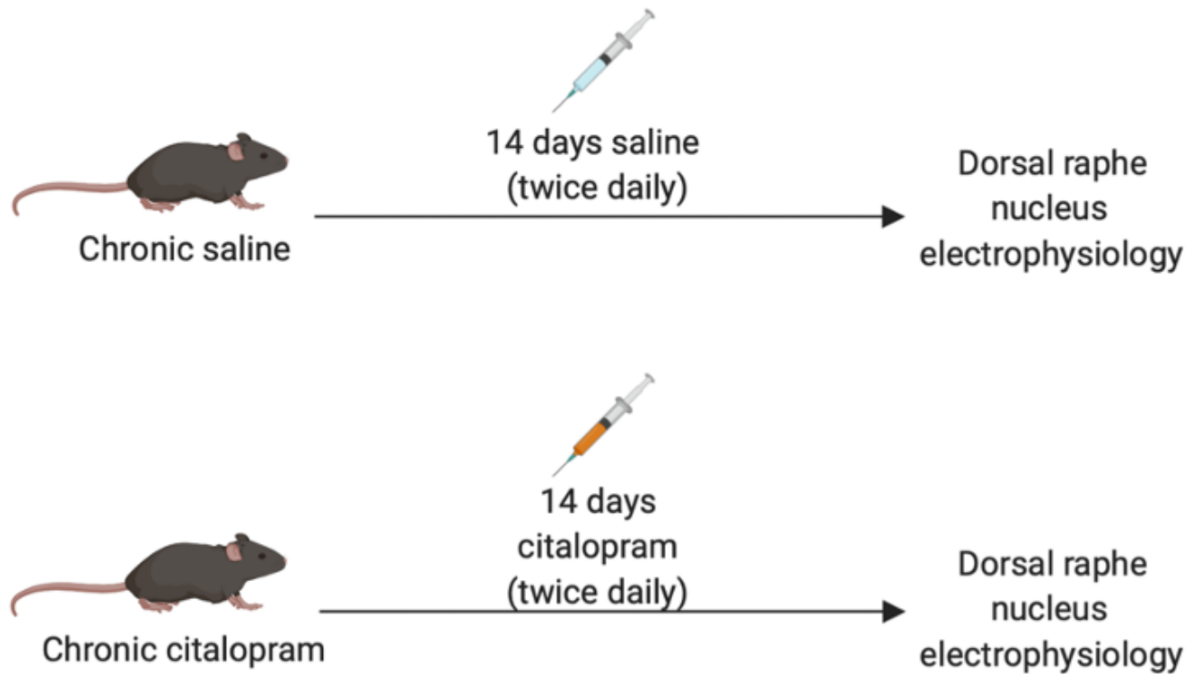
5.4.6 Conclusion

The present study utilized silicon probes to perform large-scale electrophysiological recordings of DRN neurons in mice chronically treated with the SSRI citalopram. Two populations of putative 5-HT neurons with distinct firing patterns were identified that may have been affected in different ways by citalopram treatment. It was shown that SSRI treatment attenuated neuronal correlations occurring between putative 5-HT neurons, potentially resulting from partial desensitization of 5-HT_{1A} autoreceptors. It was demonstrated that citalopram treatment enhanced the sensitivity of putative 5-HT neurons to aversive foot shock stimulus, demonstrating for the first time an effect of SSRIs on the tuning of these neurons to physiological stimuli. Finally, evidence was found that DRN GABA neurons may be tonically inhibited by continued citalopram treatment.

These results extend existing knowledge of the effects of SSRIs on the DRN 5-HT system. They indicate that SSRIs may differentially modulate the dynamics of subpopulations of 5-HT neurons, both through effects on their spontaneous firing and through alterations in their sensitivity to physiological stimuli. They also indicate that, in addition to their actions on 5-HT neurons, SSRIs may alter the dynamics of non-5-HT neurons in the DRN.

5.5 Figures and tables

5.5.1 Methods Figures



Methods Figure 5.1: Pretreatment protocol

Mice were randomly assigned into two groups, chronic saline (SAL) and chronic citalopram (CIT). Both groups received twice daily doses of citalopram for 14 days. On the 15th day, in vivo electrophysiological recordings using silicon probes in the dorsal raphe nucleus were made.

5.5.2 Results Figures

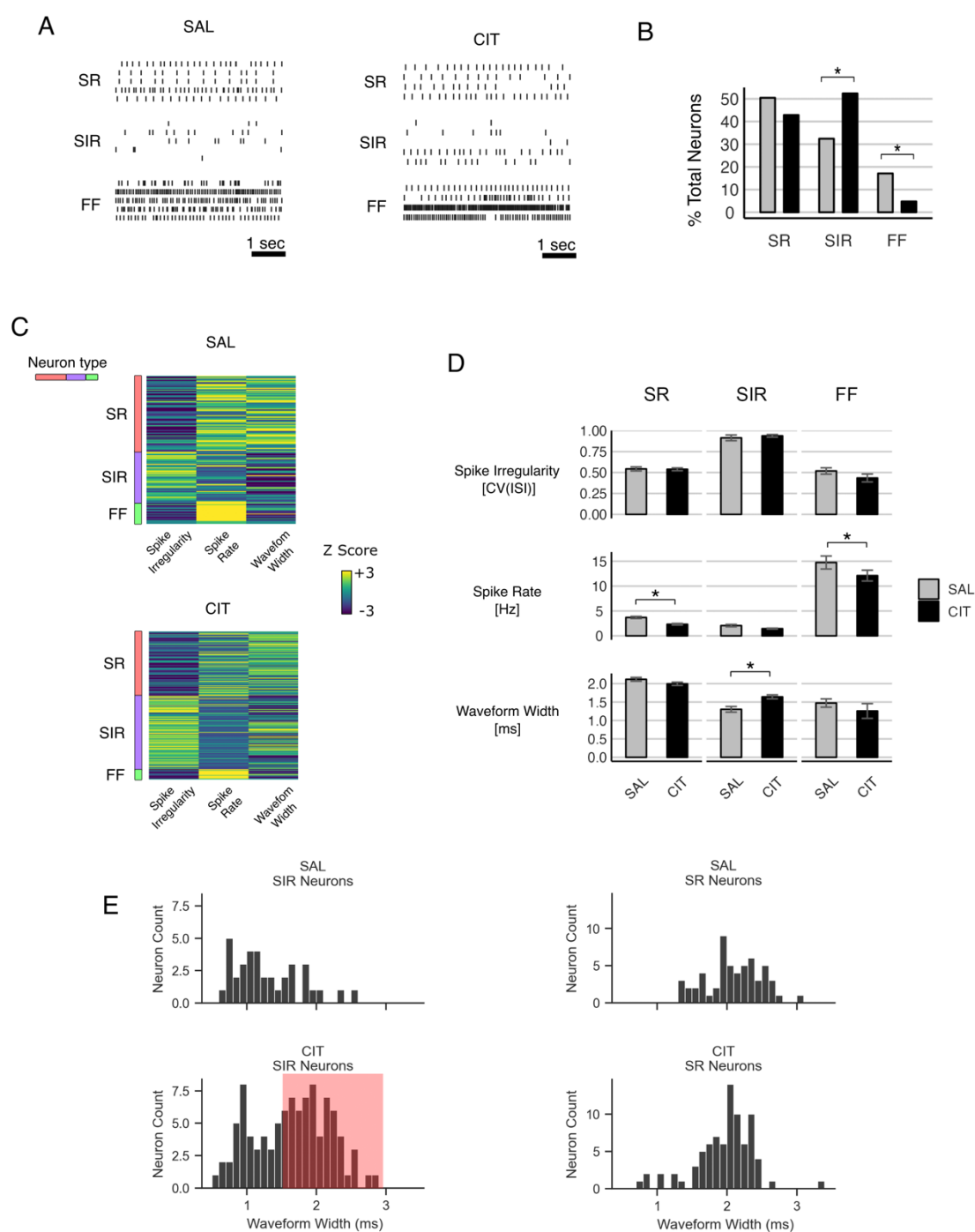


Figure 5.1: Effect of citalopram treatment on the electrophysiological properties of DRN neurons.

- A)** Raster plot from example SAL (left) and CIT (right) recordings. Each plot displays all simultaneously recorded neurons and are organized by neuron type (left).
- B)** Relative frequency of each neuron type in SAL (grey) and CIT (black) recordings. The relative frequency of SIR was higher and FF neurons was lower in CIT with respect to SAL mice.
- C)** Electrophysiological properties of individual neurons (rows) in SAL (top plot) and CIT (bottom plot) mice.
- D)** Electrophysiological properties of SR, SIR and FF neurons (see top key) by group (see color key). The spike rate of SR and FF neurons was lower in CIT with respect to SAL mice. The waveform width of SIR neurons was broader in CIT than SAL mice. Error bars cover 2 x SEM.
- E)** Distribution of waveform widths of SIR neurons in SAL and CIT mice (top and bottom respectively). A subset of SIR neurons recorded in CIT animals (red highlight) had broad waveforms typically associated with SR neurons (see right distributions of waveform widths for SR neurons in SAL and CIT mice).

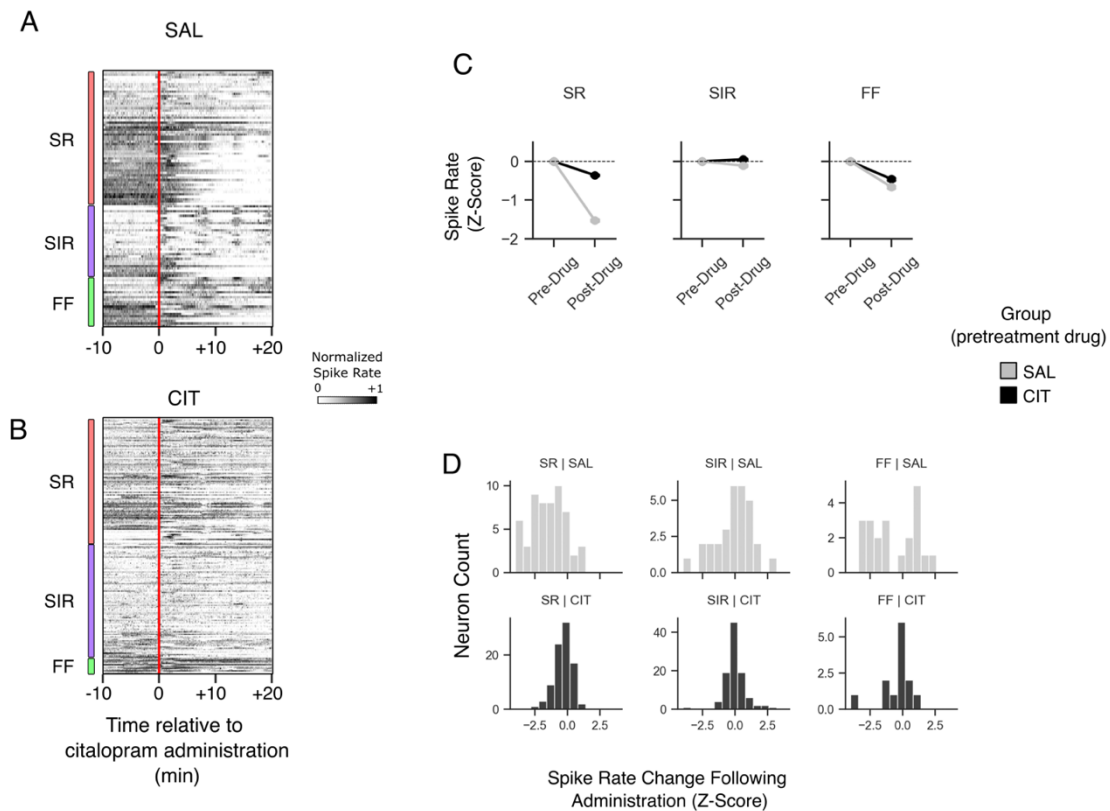


Figure 5.2: Effects of an acute citalopram challenge.

- A)** Activity of all neurons recorded in SAL mice during the pre-drug and post-drug blocks. Neurons are ordered by neuron type (see left key) and spike counts were normalized to be between 0 and 1 (see right color key).
- B)** Activity of all neurons recorded in CIT mice during the pre-drug and post-drug blocks. Neurons are ordered and normalized as in **A**.
- C)** The spike rate of neurons during the pre-drug and post-drug blocks. Values are z-scored relative to the pre-drug block. Values are organized by group (see below color key). Error bars cover 95% confidence intervals around the mean.
- D)** Distribution of changes in standardized spike rate following citalopram administration across neurons. Neurons are grouped by neuron type (column facets) and group (row facets). Histograms are colored by group (see color key above).

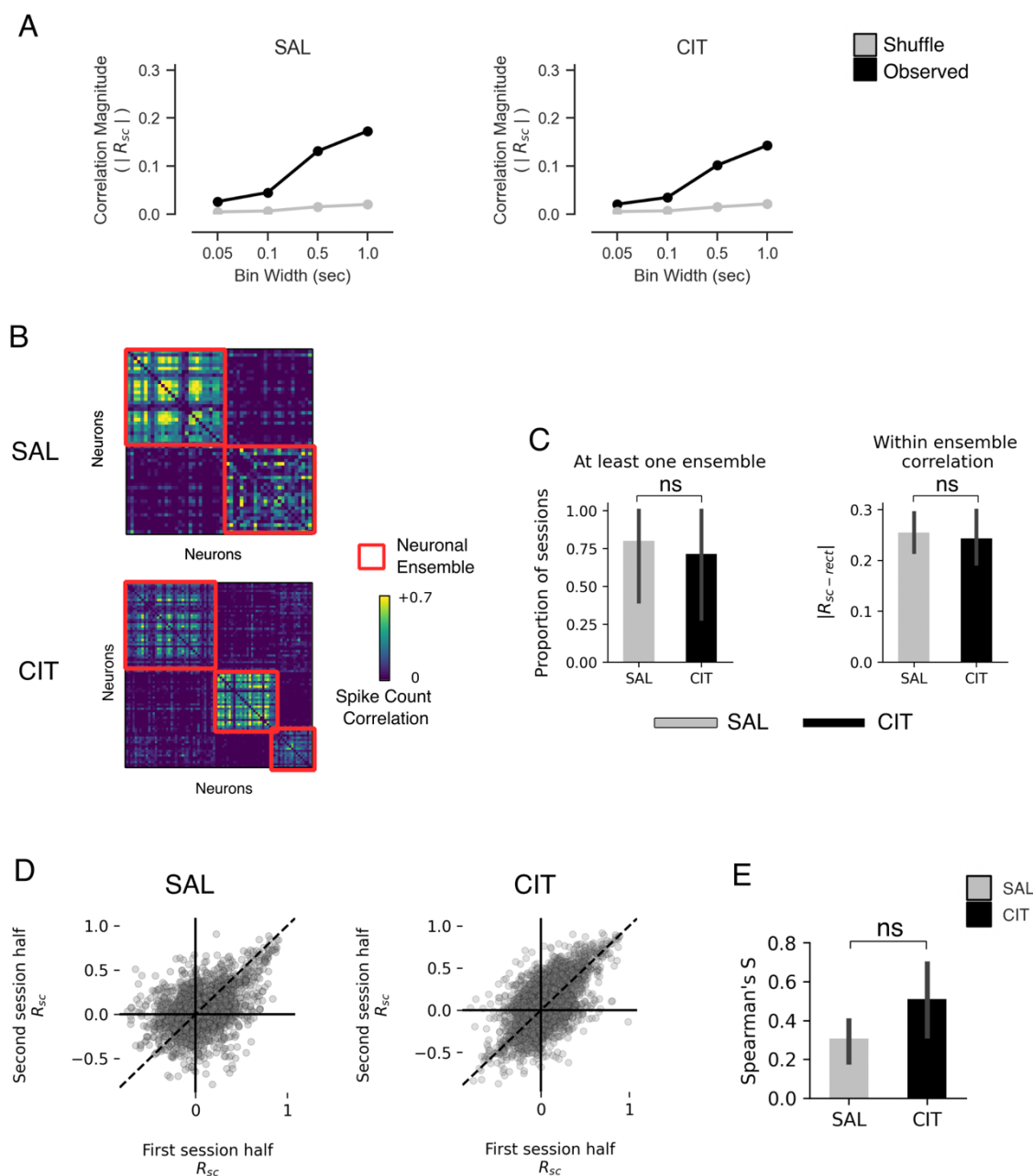


Figure 5.3: Effect of citalopram on the timescale, structure, and stability of DRN neuronal correlations.

A) Relationship between duration of the binning interval used for conversion of spike trains into timeseries of spike counts (bin width) and resultant magnitude of r_{sc} among neuron pairs recorded in SAL (left plot) and CIT (right plot) mice. Shuffle data correspond to inter-spike-interval shuffled surrogate neuron pairs. Error bars cover 95% confidence intervals around the mean.

- B)** Rectified spike count correlation matrices from an example SAL (top) and CIT (bottom) mouse. Neuronal correlations had a modular structure in both and contained neuronal ensembles (red boxes).
- C)** There was no association between group and the proportion of recording sessions having neuronal ensembles (left). There was no significant difference between the mean within ensemble rectified spike count correlation of ensembles by group. Error bars cover 95% confidence intervals around the mean.
- D)** Stability of pairwise spike count correlations (r_{sc}). Scatter plots display r_{sc} values of all neuron pairs calculated during the first and second halves of recording sessions (x and y axes, respectively). Neuron pairs appearing on the dashed line had the same r_{sc} value in both session halves. Data are plotted separately for SAL (left plot) and CIT (right plot) mice.
- E)** There was no significant difference between the first half, second half Spearman correlation coefficients SAL versus CIT sessions. Error bars cover 95% confidence intervals around the mean.

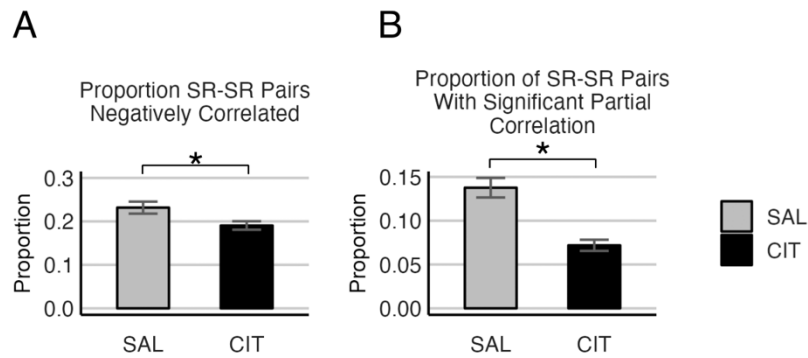


Figure 5.4: Effect of citalopram on neuronal correlations between SR-SR neuron pairs.

- A)** Proportion of SR-SR pairs with negative spike count correlation (r_{sc}) by group (see right color key). Negative correlations were less prevalent among CIT neuron pairs with respect to SAL pairs. Error bars cover 95% confidence intervals around the mean.
- B)** Proportion of SR-SR pairs with significant partial spike count correlation $r_{sc-partial}$ by group (see right color key). Significant $r_{sc-partial}$ relationships were less prevalent among CIT neuron pairs with respect to SAL pairs. Error bars cover 95% confidence intervals around the mean.

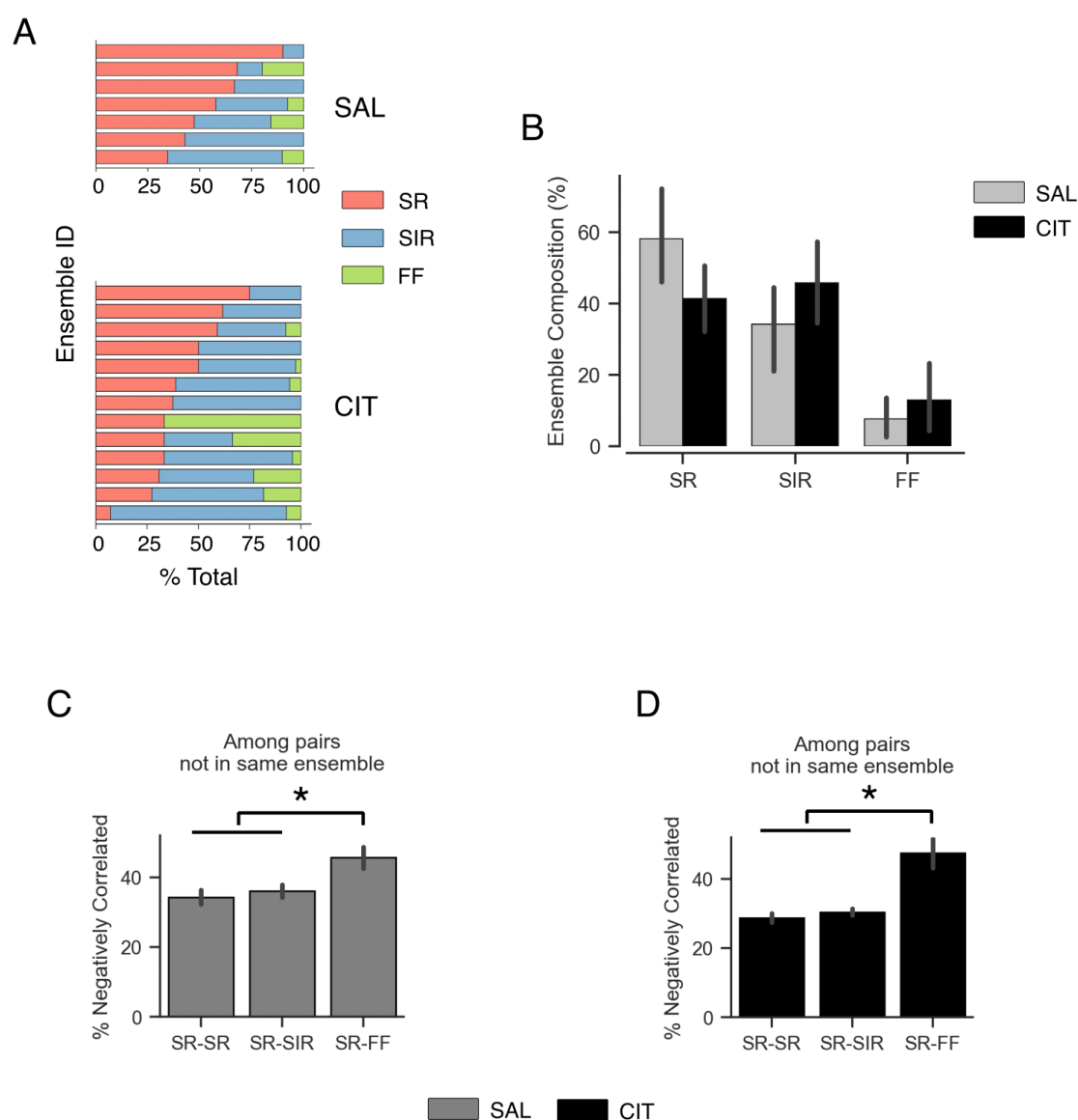


Figure 5.5: Effect of citalopram on neuronal correlations of SIR and FF neurons.

- A)** Neuron type composition of neuronal ensembles recorded in SAL (top plot) and CIT (bottom plot) mice. All neuronal ensembles (individual rows) contained multiple neuron types (colors, see right key).
- B)** Mean ensemble composition of each neuron type by group (see right color key). Error bars cover 95% confidence intervals around the mean.
- C)** Proportion of neuron pairs recorded in SAL mice that were not members of the same ensemble (not-same-ensemble neuron pairs) that had significantly negative spike count correlation coefficients. Such negative correlations were significantly

more prevalent among SR-FF neuron pairs than SR-SR and SR-SIR neuron pairs. Error bars cover 95% confidence intervals around the mean.

- D)** Proportion of not-same-ensemble neuron pairs in CIT mice that had significantly negative spike count correlations. Such negative correlations were significantly more prevalent among SR-FF neuron pairs than SR-SR and SR-SIR neuron pairs. Error bars cover 95% confidence intervals around the mean.

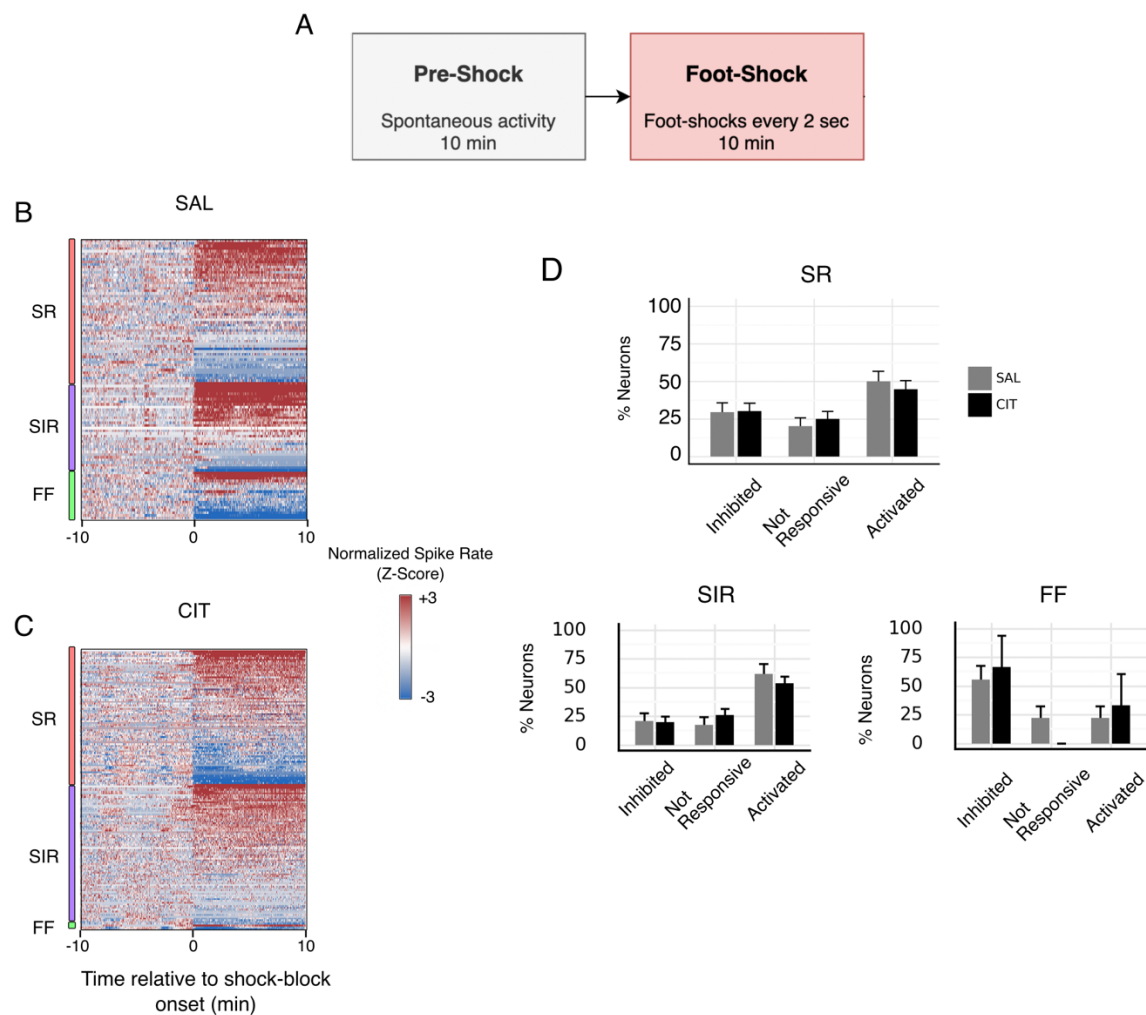


Figure 5.6: Effect of citalopram on the slow timescale response profiles of DRN neurons to foot-shocks.

- A)** Schematic illustrating the protocol for delivery of foot-shocks. The slow timescale response profiles of each neuron were classified as shock-activated, shock-inhibited, or non-responsive through comparison of their firing activity during the pre-shock block to the foot-shock block.
- B)** Neuronal activity of all neurons recorded in SAL mice during the pre-shock and post-shock blocks. Neurons are ordered according to neuron type (see left color key). Neuronal activity is standardized (see right color key).
- C)** Neuronal activity of all neurons recorded in CIT mice during the pre-shock and post-shock blocks. Neurons are ordered according to neuron type (see left color key). Neuronal activity is standardized (see right color key).

D) Distribution of slow timescale foot-shock response profiles by group (see right color key). Neurons are facettted by neuron type (see plot titles). There was no association between response profile and group for any neuron type. Error bars cover 95% confidence intervals around the mean.

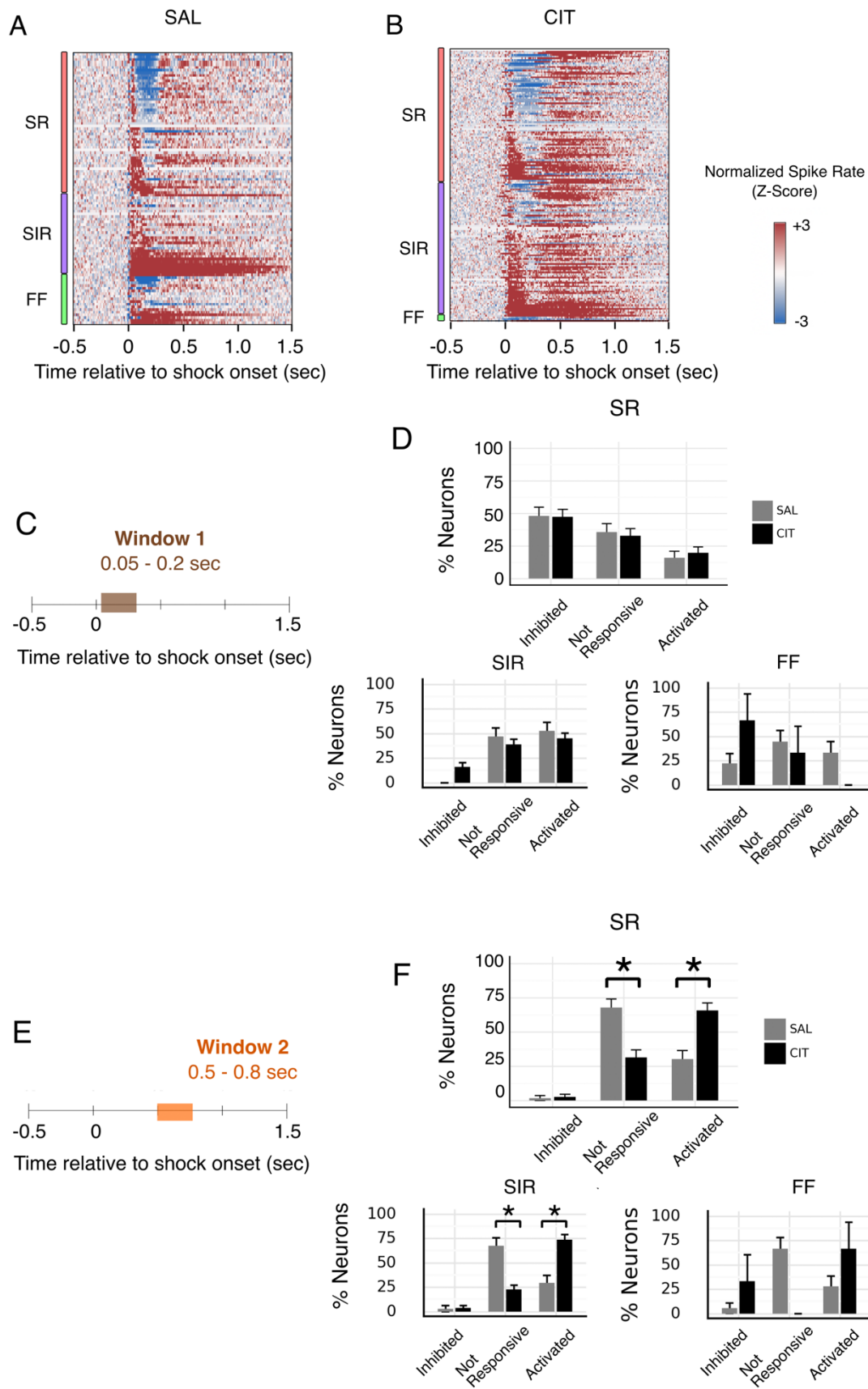


Figure 5.7: Effect of citalopram on fast timescale response profiles of DRN neurons to foot-shocks.

- A)** Peri-stimulus time histograms (PSTHs) of all neurons recorded in SAL mice. Neurons are ordered according to neuron type (see left color key). Neuronal activity is standardized (see right color key).
- B)** PSTHs of all neurons recorded in CIT mice. Neurons are ordered and standardized as in **A**.
- C)** Schematic illustrating the initial window assessed for fast timescale effects ('window 1'). Neurons were classified as shock-activated, shock-inhibited or non-responsive through comparison of their firing activity during a 0.05 – 0.2 sec window with other timepoints relative to shock onset.
- D)** Distribution of foot-shock response profiles when computed using the initial window. Neurons are plotted by group (see right color key) and are faceted by neuron type (see plot titles). There was no association between response profile and group for any neuron type. Error bars cover 95% confidence intervals around the mean.
- E)** Schematic illustrating the second window assessed for fast timescale effects ('window 2'). Neurons were classified as shock-activated, shock-inhibited or non-responsive through comparison of their firing activity during a 0.5 – 0.8 sec window with other timepoints relative to shock onset.
- F)** Distribution of foot-shock response profiles when computed using the second window. Neurons are plotted as in **D**. A greater proportion of SR and SIR neurons were activated in CIT versus SAL mice. A lower proportion of SR and SIR neurons were not responsive in CIT versus SAL mice. Error bars cover 95% confidence intervals around the mean.

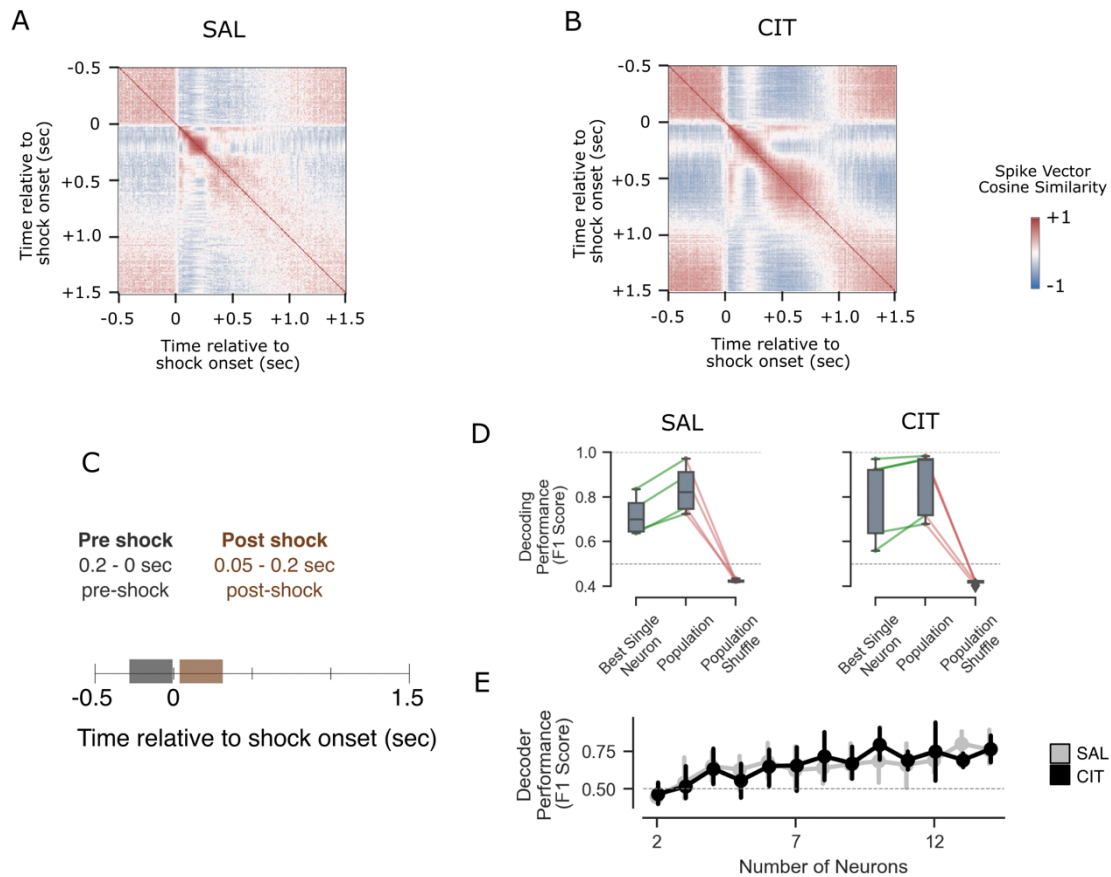


Figure 5.8: Decoding analysis of foot-shock status.

- A)** Similarity map of population activity of all SAL neurons at time points relative to shock onset. Color corresponds to cosine similarity of population activity at time points indicated at x and y axes.
- B)** Similarity map as in **A** but using data from CIT neurons.
- C)** Schematic of the decoding objective. Decoders predicted whether neuronal activity occurred during a 0.2 – 0 sec pre shock window or a 0.05 – 0.2 sec post shock window.
- D)** Performance of decoders in SAL (left plot) and CIT (right plot) mice. For both SAL and CIT mice, population decoders had higher performance than both single neuron decoders and shuffle controls.
- E)** Decoder performance as a function of the number of neurons used to make predictions. For both SAL and CIT mice (see color key), decoder performance

improved with increasing numbers of neurons. Error bars cover 95% confidence intervals around the mean.

5.5.3 Tables

GROUP	SR	SIR	FF
SAL	81	53	21
CIT	119	135	17

Table 5.1: Number of neurons of each neuron type by group.

NEURON TYPE COMBINATION	SAL	CIT
SR-SR	933	1625
SR-SIR	1161	3227
SR-FF	435	247

Table 5.2: Number of neuron pairs by neuron type combination and group.

NEURON TYPE COMBINATION	SAL	CIT	ODDS RATIO	P
SR-SR	23.17 $\pm$ 1.39	19.06 $\pm$ 0.97	1.28 $\pm$ 0.13	0.0136
SR-SIR	23.19 $\pm$ 1.24	19.44 $\pm$ 0.7	1.25 $\pm$ 0.1	0.0066
SR-FF	28.7 $\pm$ 2.17	26.79 $\pm$ 2.82	1.1 $\pm$ 0.2	0.5942
SIR-SIR	17.13 $\pm$ 2.01	18.46 $\pm$ 0.93	0.91 $\pm$ 0.14	0.5577
SIR-FF	35.5 $\pm$ 2.91	32.21 $\pm$ 2.52	1.16 $\pm$ 0.2	0.3919
FF-FF	24.25 $\pm$ 6.36	41.81 $\pm$ 8.58	0.45 $\pm$ 0.22	0.1017

Table 5.3: Comparison of negative spike count correlations by neuron type combination.

Values correspond to mean percentage $\pm$ SEM.

NEURON TYPE COMBINATION	SAL	CIT	ODDS RATIO	P
SR-SR	13.77 $\pm$ 1.12	7.19 $\pm$ 0.64	2.06 $\pm$ 0.27	0.00
SR-SIR	13.47 $\pm$ 1.01	9.85 $\pm$ 0.52	1.43 $\pm$ 0.15	0.00
SR-FF	21.21 $\pm$ 1.96	26.08 $\pm$ 2.79	0.76 $\pm$ 0.14	0.15
SIR-SIR	22.53 $\pm$ 2.2	12.59 $\pm$ 0.79	2.02 $\pm$ 0.29	0.00
SIR-FF	21.9 $\pm$ 2.48	22.36 $\pm$ 2.25	0.97 $\pm$ 0.19	0.89
FF-FF	50.73 $\pm$ 7.45	68.48 $\pm$ 8.04	0.47 $\pm$ 0.23	0.00

Table 5.4: Comparison of partial spike count correlations by neuron type combination.

Values correspond to mean percentage $\pm$ SEM.

Chapter 6: General discussion

6.1 Background and summary

Work performed in this thesis applied silicon probe electrode recording technology to investigate the electrophysiology of the DRN 5-HT system. Experiments were directed towards quantifying the diversity of firing properties among putative 5-HT and non-5-HT neurons in the DRN, assessing for the presence of emergent population dynamics such as neuronal ensembles and population coding, and investigating the effect on these parameters of an aversive foot shock stimulus and treatment with the SSRI citalopram.

In Chapter 2 the silicon probe recording method used in all subsequent chapters was established. It was found that silicon probes could be targeted to the DRN and generate simultaneous recordings of multiple DRN neurons. Through clustering analysis recorded neurons could be characterized according to three predominant firing patterns: slow-regular, slow-irregular, and fast-firing neurons (SR, SIR, and FF neurons). The properties of SR, SIR, and FF neurons were consistent with those of 5-HT, generic non-5-HT, and GABA neurons respectively, on the basis of the prevailing literature on juxtacellular labelling (Allers & Sharp, 2003; Schweimer et al., 2011; Schweimer & Ungless, 2010).

Chapter 3 applied the silicon probe recording method to assess the effect of an aversive foot shock stimulus on the firing of DRN neurons. It was found that foot shocks had complex effects on the firing of SR neurons which occurred over multiple timescales. Thus, many SR neurons responded with both changes in firing that were time-locked to stimulus onset (fast timescale effects) and altered firing rates during a

10-min block when foot shocks were repeatedly presented (slow timescale effects). Diverse responses of SR neurons were noted on both timescales with some neurons being activated, others inhibited and yet others being non-responsive to the foot shock stimulus. Widespread and diverse responsivity to foot-shocks was also detected among putative non-5-HT neurons (i.e., SIR and FF neurons) on both timescales. Analysis of foot-shock stimulus decoders found improved performance as a function of increasing number of neurons used to make predictions, consistent with DRN neurons encoding the stimulus using a population code (Nicolelis & Lebedev, 2009).

Chapter 4 leveraged the simultaneous nature of silicon probe recordings to investigate neuronal interactions within the DRN network. Analysis of pairwise spike count correlations indicated that the majority of DRN neuron pairs were correlated on slow but not fast timescales, i.e., many neurons had correlated spike rates but not synchronized spike timings. Interactions between pairs of SR neurons were heterogeneous with many neurons being highly correlated in a positive manner, while the activity of others was either negatively correlated or independent of each other. Highly correlated SR neurons formed neuronal ensembles, groups of intercorrelated neurons with correlated fluctuations in spike rates (Carillo-Reid & Yuste, 2020; Humphries, 2018). Correlations between SR neurons and SIR and FF neurons were also heterogeneous; the latter neurons could be members of the same neuronal ensembles as SR neurons but when they were not these neurons were often negatively correlated, especially for (putative GABA) FF neurons.

Chapter 5 assessed the effect of repeated treatment with the SSRI citalopram on the baseline neuronal firing of DRN neurons and their sensitivity to an aversive foot shock. Evidence was found that subpopulations of 5-HT neurons may be affected in

different ways by citalopram. Thus, two populations of putative 5-HT neurons were identified in citalopram-treated animals, one population of broad waveform, regular firing (SR) neurons that had reduced firing rates with respect to saline controls, and another population of broad waveform neurons with more irregular firing patterns. It was found that SR neurons in citalopram-treated animals had enhanced excitability to foot shocks, identifying for the first time an effect of SSRI treatment on the sensitivity of putative 5-HT neurons to a physiological stimulus. Evidence was found that repeated citalopram also impacted on non-5-HT neurons in the DRN. In particular, FF neurons had a reduced prevalence in citalopram-treated mice, and those FF neurons that were observed had lower firing rates than those recorded in saline controls.

6.2 5-HT neuron diversity

As detailed in Chapter 1, recent neuroanatomical and transcriptomic studies have reported considerable heterogeneity among 5-HT neurons: clusters of 5-HT neurons have been identified that, with respect to neighboring 5-HT neurons in the DRN, have distinct anatomical connectivity, patterns of gene expression and developmental lineage (Commons, 2015; Huang et al., 2019; Niederkofler et al., 2016; Okaty et al., 2019; Ren et al., 2018, 2019; Weissbourd et al., 2014). Up until now it has been poorly understood how this heterogeneity is expressed in the firing of DRN 5-HT neurons, in part due to the low yield of the single unit electrodes that have typically been used to monitor DRN neurons (Allers & Sharp, 2003; Jacobs & Azmitia, 1992; Schweimer & Ungless, 2010; Urai et al., 2021; Varga et al., 2003). A main aim of this thesis was to apply large-scale recordings to identify the extent of diversity in the electrophysiological properties of DRN 5-HT neurons. Important findings in this

regard included (1) the quantification in Chapter 3 of the diversity of response profiles of putative 5-HT neurons to an aversive foot-shock stimulus; and (2) the evidence identified in Chapter 5 that repeated citalopram treatment may impact on the firing of subpopulations of 5-HT neurons in different ways.

This diversity among putative 5-HT neurons with respect to their sensitivity to citalopram and foot-shocks is consistent with recent reports of heterogeneity among 5-HT neurons with respect to their gene expression profiles and anatomical connectivity (Okaty et al., 2019).

As detailed in Chapters 1 and 5, acute administration of SSRIs inhibits the firing of 5-HT neurons indirectly by elevating levels of extracellular 5-HT in the DRN to activate 5-HT_{1A} autoreceptors, with the latter becoming desensitized with continued SSRI treatment (Bel & Artigas, 1992; Blier & de Montigny, 1994; Blier & El Mansari, 2013; Czachura & Rasmussen, 2000; Mansari et al., 2005). Recent studies have reported diversity among 5-HT neurons with respect to their expression levels of 5-HT receptors including 5-HT_{1A} autoreceptors (Huang et al., 2019; Kirby et al., 2003; Marinelli et al., 2004; Ren et al., 2019). This latter finding may account for the observation in Chapter 5 of two populations of putative 5-HT neurons that had different firing characteristics following continued citalopram treatment (slow firing broad waveform neurons with regular and irregular firing patterns): these neuronal populations may have been 5-HT neurons that were differentially sensitive to citalopram due to varying levels of expression of 5-HT_{1A} autoreceptors.

Heterogeneity of responses of DRN 5-HT neurons to aversive foot shock is in accord with reports that these neurons vary in terms of their inputs from brain regions involved in aversive processing. For example, the PFC and LHb have both been linked

to regulating the activity of 5-HT neurons during aversive processing (Amat et al., 2005; Dolzani et al., 2016; Maier, 2015; M. R. Warden et al., 2012), and recent neuroanatomical studies have identified that the inputs of these regions to the DRN target specific subpopulations of 5-HT neurons (Sego et al., 2014; Weissbourd et al., 2014). Thus, the different ways in which putative 5-HT neurons respond to aversive foot-shock could be due to their differential inputs from the LHb or PFC.

Interestingly, there is evidence that subpopulations of 5-HT neurons can impact on behavioral outputs and cognitive states in different ways (Niederkofler et al., 2016; Ren et al., 2018). As discussed below, an important area of future investigation will be to link the diversity of firing properties of DRN neurons identified in this thesis with diversity in the functional properties of these neurons.

6.3 5-HT neurons functioning as a population and as part of a network

5-HT neurons were originally thought to be homogenous interchangeable units that together generated a unified 5-HT signal (Gaspar & Lillesaar, 2012; Jacobs & Azmitia, 1992). The recent evidence for heterogeneity amongst DRN 5-HT neurons of the sort noted above has prompted an alternative idea, namely that the 5-HT system may be comprised of several functionally distinct submodules (Abrams et al., 2004; Calizo et al., 2011; Huang et al., 2019; Okaty et al., 2019; Ren et al., 2019). An important aim of this thesis was to investigate how DRN 5-HT neurons function as a population by leveraging the capacity of silicon probes to monitor multiple neurons simultaneously.

Experiments in Chapter 4 identified both interactions between pairs of putative 5-HT neurons and the presence of neuronal ensembles within the DRN network. These

findings are in accord with a recent study that found similar results employing one-photon calcium imaging to monitor DRN 5-HT neurons in awake behaving mice (Paquelet et al., 2022). Work in this thesis demonstrated the temporal stability of these neuronal ensembles: the structure of neuronal correlations in the DRN was similar over the course of a recording session, consistent with the composition of neuronal ensembles being relatively static.

This evidence of modular, heterogeneous interactions between 5-HT neurons is in line with the reported heterogeneity amongst 5-HT neurons noted above (Abrams et al., 2004; Commons, 2015; Weissbourd et al., 2014). For example, the current evidence that the dynamics of DRN 5-HT neurons within an ensemble tends to be more highly correlated compared to non-ensemble neurons fits well with the findings of one recent large scale neuroanatomical analysis that reported that clusters of 5-HT neurons had distinct patterns of connectivity with respect to neighboring 5-HT neurons in the DRN (Commons, 2015). Also, other studies have identified topographic variation among DRN 5-HT neurons with respect to their expression of neurotransmitter receptors including corticotrophin releasing hormone receptors (Abrams et al., 2004).

The decoding analysis of Chapter 3 also informs on the functioning of DRN 5-HT neurons as a population and as part of a network. It was found that decoding models of foot-shock status performed poorly when the predictions were made from the activity of single DRN neurons but improved markedly with increasing numbers of DRN neurons used by decoders. These results are similar to those of studies that compared the performance of single neuron and population decoders in various forebrain regions including visual and auditory sensory cortex, motor cortex and the PFC (Georgopoulos et al., 1986; Gutierrez et al., 2006; Hung et al., 2005; Katz et al.,

2002; Laubach et al., 2000; Nicolelis & Lebedev, 2009; Rigotti et al., 2013; Runyan et al., 2017; Simon et al., 2006; Stringer et al., 2021). These studies have interpreted the improved performance of population decoders as evidence that neurons in these regions were employing a population coding scheme, that is a neural coding framework that posits that cognitive and behavioral variables are encoded in the joint activity of multiple neurons rather than single neurons alone (Nicolelis & Lebedev, 2009; Saxena & Cunningham, 2019; Yuste, 2015). Thus, the decoding analysis of Chapter 3 provides evidence that DRN neurons, including 5-HT neurons, encode aversive stimuli using a population code (though see later discussion).

Population coding of aversive stimuli by the DRN 5-HT system could serve several functions. Population coding could act to decrease the variance of responses to aversive stimuli. Thus, although many single neurons had tuning to the foot-shock stimulus, downstream neurons may decode aversive state by performing a weighted average of responses across groups of neurons. There is evidence from both theoretical and experimental studies that such a population coding approach can reduce the trial-by-trial variability of evoked activity in neuronal populations, so long as trial by trial variability is not correlated between neurons (Kohn et al., 2016; Stringer et al., 2021).

Another potential scenario is that DRN neurons employ a population code to signal multiple cognitive and behavioral variables in distinct ways. As outlined in Chapter 1 there is evidence that the dynamics of DRN neurons, including 5-HT neurons, are tuned to a large number of physiological stimuli and cognitive states as well as behavioral outputs related to emotionality (Cohen et al., 2015; Dayan & Huys, 2009; Li et al., 2016b; Marcinkiewicz et al., 2016; Sengupta & Holmes, 2019). Furthermore,

and again as outlined in Chapter 1, theoretical work indicates that, assuming both that activity is read out by linear decoders and neurons exhibit non-linear mixed selectivity, the representational capacity of neuronal populations is larger when neuronal populations employ population codes (Fusi et al., 2016; Kaufman et al., 2022).

Overall, work in this thesis identified two important findings regarding the functioning of 5-HT neurons as a population and as part of a network. First, evidence was found that 5-HT neurons could be members of neuronal ensembles, which were identified for the first time in the DRN using electrophysiological methods. Second, evidence was identified that 5-HT neurons encode an aversive foot-shock stimulus through their joint activity by contributing to a DRN population code.

6.4 Non-5-HT neurons in the DRN

DRN 5-HT neurons are embedded amongst many non-5-HT neurons including GABA, glutamate, dopamine, and peptide neurons (Belin et al., 1979; Gamrani et al., 1979; Grzanna & Molliver, 1980; Kaneko et al., 1990; Ochi & Shimizu, 1978). Despite evidence from pharmacological, neuroanatomical, and behavioral studies that indicates that these neurons can both influence the activity of local 5-HT neurons (Boothman & Sharp, 2005; Sharp et al., 2007; Varga et al., 2001, 2003; Weissbourd et al., 2014), and act as projection neurons in their own right (Qi et al., 2014; Xu et al., 2021), few electrophysiological studies of the DRN have reported on their firing properties. An important aim of this thesis was to apply silicon probe recordings to investigate the firing properties of putative non-5-HT DRN neurons, leveraging the capacity of these electrodes to monitor large numbers of neurons in a cell type agnostic manner.

This thesis investigated the effect of an aversive foot-shock stimulus and treatment with the SSRI citalopram on the dynamics of SIR and FF neurons (putative generic non-5-HT and GABA neurons, respectively). These two perturbations are well known to modulate the activity of DRN 5-HT neurons (Blier & El Mansari, 2013; Cohen et al., 2015; Mansari et al., 2005; Schweimer & Ungless, 2010) but their effects on local non-5-HT neurons are little investigated. It was found that the dynamics of many SIR and FF neurons were altered by both citalopram treatment and the foot-shock stimulus. Thus, experiments reported in Chapters 2 and 5 found that FF neurons were inhibited by acute and chronic citalopram. Moreover, experiments in Chapter 3 identified that both SIR and FF neurons exhibited widespread responsivity to foot-shocks.

Notably, foot shocks and citalopram often impacted on the firing of SIR and FF neurons in different ways to their effects on SR neurons. Thus, while both SR and FF neurons were inhibited by acute citalopram in Chapter 2, subsequent administration of a 5-HT_{1A} receptor antagonist reversed SR neuron inhibition but had no effect on the activity of FF neurons. Furthermore, while both SR and SIR neurons were sensitive to foot-shocks, many SR neurons had short latency inhibitions while the most common response of SIR neurons was short latency excitations. These results add support to SR, SIR and FF neurons being distinct neuronal populations.

Non-5-HT neurons being sensitive to citalopram and aversive stimuli is consistent with the findings of several studies that have investigated their neurobiological properties. As outlined in Chapter 1 and above, afferent input arising from the LHb, PFC, and nucleus raphe magnus is thought to control the activity of DRN neurons during aversive processing (Dolzani et al., 2016; Maier, 2015; Q. P. Wang & Nakai,

1994). Neuroanatomical studies have revealed that input from these brain regions to the DRN can synapse on both glutamate and GABA neurons (Weissbourd et al., 2014; Xu et al., 2021). Similarly, putative GABA neurons being sensitive to SSRIs, which increase extracellular concentrations of 5-HT in the DRN (Bel & Artigas, 1992), is in line with pharmacological and transcriptomic studies that have identified expression of functional 5-HT receptors on GABA neurons (Boothman & Sharp, 2005; Huang et al., 2019; Sharp et al., 2007).

The function of non-5-HT neurons in the DRN response to aversive stimuli or citalopram treatment is not clear. One possibility is that the primary function of non-5-HT neurons is to control the activity of local 5-HT neurons. Thus, non-5-HT neurons that are modulated by aversive stimuli may act to regulate the stimulus evoked activity in local 5-HT neurons. Similarly, the primary consequence of SSRI actions on non-5-HT neurons could be changes in how they regulate the activity of local 5-HT neurons. Consistent with this idea, there is evidence for pharmacological manipulations of DRN GABA neuron activity having indirect consequences on the activity of 5-HT neurons (Boothman & Sharp, 2005; Sharp et al., 2007) as well as for DRN GABA neurons feeding input from the LHb and PFC onto local 5-HT neurons (Hajós et al., 1998; Varga et al., 2001, 2003). Thus, the changes in FF neuron firing noted in response to citalopram and foot-shocks may impact on the firing of local 5-HT neurons.

Another possible scenario is that non-5-HT DRN neurons are projection neurons. Thus, the primary action of these neurons may be independent of local 5-HT neurons. This idea is supported by neuroanatomical studies that have identified projections of non-5-HT neurons including glutamate and GABA neurons out of the DRN to several

forebrain sites linked to emotionality including the VTA, PFC, and amygdala (Qi et al., 2014; Xu et al., 2021).

Finally, it is also possible that non-5-HT DRN neurons are sensitive to SSRI treatment and aversive stimuli because they are responding to changes in the dynamics of local 5-HT neurons. Consistent with this idea, 5-HT neurons project within the DRN to target non-5-HT neurons (Adell et al., 2002; Weissbourd et al., 2014). Also, and as noted above, non-5-HT DRN neurons express 5-HT receptors and have been reported to have altered activity following the administration of 5-HT and 5-HT receptor agonists (Boothman et al., 2006; Huang et al., 2019; R. Liu et al., 2000; Sharp et al., 2007).

6.5 Future work

6.5.1 Verification of DRN neuron types

Work in this thesis leans on previous evidence from juxtacellular and intracellular labeling studies that SR, SIR, and FF neurons are respectively 5-HT, non-5-HT, and GABA neurons (Aghajanian et al., 1978; Allers & Sharp, 2003; Schweimer et al., 2011; Schweimer & Ungless, 2010). However, data from these latter studies were not binary. For example, several studies have reported slow regular firing neurons that were immunonegative for markers of 5-HT neurons as well as fast-firing neurons that were immunopositive for these 5-HT markers (Allers & Sharp, 2003; Kocsis et al., 2006; Schweimer & Ungless, 2010).

Future studies could build upon the work of this thesis by employing optogenetic tagging to estimate the neurochemical identity of recorded neurons. This approach is based upon targeting a light sensitive cation channel to a genetically defined neuronal

population such as 5-HT neurons and then identifying them by their responsivity to light emissions. The primary advantage of optogenetic tagging is that it is thought to be associated with a very small false positive misclassification rate. Thus, future studies could employ this method to verify the identity of 5-HT, GABA, dopamine, and also glutamate neurons within the recordings. Such an experiment would likely be feasible as several recent studies have applied this method to identify 5-HT neurons in the DRN (Cohen et al., 2015; Li et al., 2016b; Z. Liu et al., 2014).

6.5.2 Anesthesia

One methodological factor that may have impacted on the measurements made in this thesis was that electrophysiological recordings were made in mice under urethane anesthesia. Several studies have measured neuronal activity during anesthesia and other brain states and have reported differences in various aspects of neuronal dynamics (Ecker et al., 2014; N. Wang et al., 2010; Yatziv et al., 2020). Two findings that are particularly pertinent are (1) a report that neuronal responses to aversive stimuli are diminished under anesthesia with respect to wakefulness (N. Wang et al., 2010), and (2) a study that demonstrated that neuronal correlations in visual cortex were inflated under anesthesia with respect to wakefulness (Ecker et al., 2014).

Despite these findings, we believe that it is likely that many of the major results in this thesis were not simply an artifact of the anesthetic setting. Performing measurements under anesthesia has been a common practice in electrophysiological studies of the DRN [e.g., (Allers & Sharp, 2003; Hajós et al., 2007; Varga et al., 2001, 2003)] and many findings initially made under anesthesia have subsequently been confirmed in awake animals. For example, the diverse responses of DRN 5-HT neurons to an aversive stimulus was initially identified under urethane anesthesia (Schweimer &

Ungless, 2010) and was subsequently confirmed in awake, head-fixed mice (Cohen et al., 2015). That the findings of this thesis relating to neuronal correlations were not simply a function of the anesthetic setting is supported by a study that identified similar features of DRN interactions in awake animals. Thus, as discussed above, the presence of heterogeneous and modular interactions among DRN 5-HT neurons was reported by a recent calcium imaging study in awake behaving mice (Paquelet et al., 2022).

Future work could extend this thesis by performing similar electrophysiological measurements in awake animals. For example, the state dependence of neuronal correlations and sensitivity to an aversive stimulus could be assessed by repeating the experiments of Chapters 3 and 4 in awake head fixed mice.

6.5.3 5-HT neuron diversity

An important area of future investigation will be to link the heterogeneous firing properties of putative 5-HT neurons identified in the present thesis to the diverse anatomical and molecular properties of these neurons. Two methods that could be employed to enable specific subpopulations of neurons to be identified are antidromic stimulation and optotagging. One application of such approaches could be to assess whether SR neurons that are activated by foot-shocks project to different brain regions compared to SR neurons that are inhibited or non-responsive. Another could be to investigate whether 5-HT neurons with specific gene expression profiles are differentially affected by continued SSRI treatment.

6.5.4 Population coding

It is important to note that decoding analyses alone cannot determine whether DRN 5-HT neurons encode aversive stimuli using a population code (Ritchie et al., 2017). One approach that has been used to further investigate whether neurons in other brain regions encode stimuli with a population code has been to compare the correlations of single neuron and population decoders to behavioral performance (Laubach et al., 2000; Nicolelis & Lebedev, 2009). For example, one study suggested that neurons in rat motor cortex employ a population code by demonstrating more tight coupling between the performance of population decoders to behavioral performance than single neuron decoders (Laubach et al., 2000). One way that this approach could be applied to the DRN is by comparing the performance of population and single neuron decoders during a task where animals learn to associate a stimulus with a punishment over several days. A tighter coupling of DRN 5-HT neuron population decoders to behavioral output would serve as evidence for these neurons employing a population code.

6.5.5 Ensembles

A key finding of this thesis is the presence of neuronal ensembles in the DRN. However, it was not known whether these ensembles constitute the functional submodules that have been predicted in several recent theories of 5-HT neuron signaling (Abrams et al., 2004; Commons, 2015; Okaty et al., 2019; Ren et al., 2019). Future work to address this issue could follow a similar approach to that used in a series of recent studies that have identified and characterized neuronal ensembles in another subcortical neuromodulatory system: noradrenergic neurons of the LC. Following the identification of neuronal ensembles in the LC (Totah, 2018), a series

of experiments demonstrated links between the anatomical and electrophysiological properties of LC neurons and ensemble membership. Thus, it was found that LC neuronal ensembles generally contained neurons with similar electrophysiological characteristics, forebrain projections, and sensitivity to behavioral outputs (Totah, 2018; Totah et al., 2019). Further work additionally found that periods of elevated activity in distinct LC ensembles were associated with distinct patterns of cortical activity (Shahryar Noei et al., 2022).

6.6 Conclusion

The present thesis utilized silicon probe recordings to investigate the electrophysiological properties of 5-HT and non-5-HT neurons within the DRN. Analyses were directed towards both characterizing the properties of individual neurons as well as quantifying relationships between the dynamics of neurons in the DRN network. A method for silicon probe recordings was established and shown to generate large scale simultaneous recordings of DRN neurons.

Considerable diversity was identified in the firing properties of putative 5-HT neurons. Evidence was found for heterogeneity at multiple levels: interactions of these neurons with neighboring putative 5-HT neurons, responsivity to an aversive foot shock stimulus, and sensitivity to treatment with the SSRI citalopram. For the first time, putative non-5-HT neurons in the DRN were also found to respond to both the foot shock stimulus and citalopram treatment.

Emergent population dynamics were detected in the DRN network. It was found that highly correlated DRN neurons formed modular neuronal ensembles that likely contained both 5-HT and non-5-HT neurons. Additionally, evidence was found for

DRN 5-HT neurons employing a population coding scheme to encode aversive stimuli, as indicated by improved performance of foot shock decoding models with increased numbers of neurons.

These findings extend existing knowledge on 5-HT neuron diversity, the functioning of non-5-HT neurons within the DRN, and the ways by which 5-HT neurons function as part of the DRN network.

References

- Abrams, J. K., Johnson, P. L., Hollis, J. H., & Lowry, C. A. (2004). Anatomic and functional topography of the dorsal raphe nucleus. *Annals of the New York Academy of Sciences*, 1018, 46–57. <https://doi.org/10.1196/annals.1296.005>
- Adell, A., Casanovas, J., Casanovas, J., Artigas, F., & Artigas, F. (1997). Comparative study in the rat of the actions of different types of stress on the release of 5-HT in raphe nuclei and forebrain areas. *Neuropharmacology*, 36(4), 735–741. [https://doi.org/10.1016/S0028-3908\(97\)00048-8](https://doi.org/10.1016/S0028-3908(97)00048-8)
- Adell, A., Celada, P., Abellán, M. T., Artigas, F., & Artigas, F. (2002). Origin and functional role of the extracellular serotonin in the midbrain raphe nuclei. *Brain Research Reviews*, 39(2), 154–180. [https://doi.org/10.1016/S0165-0173\(02\)00182-0](https://doi.org/10.1016/S0165-0173(02)00182-0)
- Adler, A., Katabi, S., Finkes, I., Prut, Y., & Bergman, H. (2013). Different correlation patterns of cholinergic and GABAergic interneurons with striatal projection neurons. *Frontiers in Systems Neuroscience*, 7, 47–47. <https://doi.org/10.3389/fnsys.2013.00047>
- Aghajanian, G. K., Foote, W. E., & Sheard, M. H. (1968). Lysergic Acid Diethylamide: Sensitive Neuronal Units in the Midbrain Raphe. *Science*, 161(3842), 706–708. <https://doi.org/10.1126/science.161.3842.706>
- Aghajanian, G. K., & Vandermaelen, C. (1982a). Intracellular identification of central noradrenergic and serotonergic neurons by a new double labeling procedure. *The Journal of Neuroscience*, 2(12), 1786–1792. <https://doi.org/10.1523/jneurosci.02-12-01786.1982>

- Aghajanian, G. K., & Vandermaelen, C. P. (1982b). Intracellular recordings from serotonergic dorsal raphe neurons: Pacemaker potentials and the effects of LSD. *Brain Research*, 238(2), 463–469. [https://doi.org/10.1016/0006-8993\(82\)90124-x](https://doi.org/10.1016/0006-8993(82)90124-x)
- Aghajanian, G. K., & Wang, R. Y. (1977). Habenular and other midbrain raphe afferents demonstrated by a modified retrograde tracing technique. *Brain Research*, 122(2), 229–242. [https://doi.org/10.1016/0006-8993\(77\)90291-8](https://doi.org/10.1016/0006-8993(77)90291-8)
- Aghajanian, G. K., Wang, R. Y., Jay M. Baraban, & Baraban, J. M. (1978). Serotonergic and non-serotonergic neurons of the dorsal raphe: Reciprocal changes in firing induced by peripheral nerve stimulation. *Brain Research*, 153(1), 169–175. [https://doi.org/10.1016/0006-8993\(78\)91140-x](https://doi.org/10.1016/0006-8993(78)91140-x)
- Alejandro-García, T., Kim, S., Pérez-Ortega, J., & Yuste, R. (2022). Intrinsic excitability mechanisms of neuronal ensemble formation. *ELife*, 11, e77470. <https://doi.org/10.7554/eLife.77470>
- Allers, K. A., & Sharp, T. (2003). Neurochemical and anatomical identification of fast- and slow-firing neurones in the rat dorsal raphe nucleus using juxtacellular labelling methods in vivo. *Neuroscience*, 122(1), 193–204. [https://doi.org/10.1016/s0306-4522\(03\)00518-9](https://doi.org/10.1016/s0306-4522(03)00518-9)
- Amat, J., Baratta, M., Baratta, M. V., Paul, E. D., Bland, S. T., Watkins, L. R., & Maier, S. F. (2005). Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. *Nature Neuroscience*, 8(3), 365–371. <https://doi.org/10.1038/nn1399>
- Arnsten, A. F. T. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nature Reviews Neuroscience*, 10(6), 410–422. <https://doi.org/10.1038/nrn2648>

- Asaoka, N., Nagayasu, K., Nishitani, N., Yamashiro, M., Shirakawa, H., Nakagawa, T., & Kaneko, S. (2015). Olanzapine augments the effect of selective serotonin reuptake inhibitors by suppressing GABAergic inhibition via antagonism of 5-HT6 receptors in the dorsal raphe nucleus. *Neuropharmacology*, *95*, 261–268. <https://doi.org/10.1016/j.neuropharm.2015.03.032>
- Azmitia, E. C., & Segal, M. (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *The Journal of Comparative Neurology*, *179*(3), 641–667. <https://doi.org/10.1002/cne.901790311>
- Aznar, S., Qian, Z.-X., & Knudsen, G. M. (2004). Non-serotonergic dorsal and median raphe projection onto parvalbumin- and calbindin-containing neurons in hippocampus and septum. *Neuroscience*, *124*(3), 573–581. <https://doi.org/10.1016/j.neuroscience.2003.12.020>
- Bair, W., Zohary, E., & Newsome, W. T. (2001). Correlated Firing in Macaque Visual Area MT: Time Scales and Relationship to Behavior. *The Journal of Neuroscience*, *21*(5), 1676–1697. <https://doi.org/10.1523/jneurosci.21-05-01676.2001>
- Baker, K. G., Halliday, G. M., Hornung, J.-P., Geffen, L. B., Cotton, R. G. H., Cotton, R. G. H., Törk, I., & I. Törk. (1991). Distribution, morphology and number of monoamine-synthesizing and substance P-containing neurons in the human dorsal raphe nucleus. *Neuroscience*, *42*(3), 757–775. [https://doi.org/10.1016/0306-4522\(91\)90043-n](https://doi.org/10.1016/0306-4522(91)90043-n)
- Baker, K. G., Halliday, G. M., & Törk, I. (1990). Cytoarchitecture of the human dorsal raphe nucleus. *The Journal of Comparative Neurology*, *301*(2), 147–161. <https://doi.org/10.1002/cne.903010202>

- Bakshi, A., & Tadi, P. (2023). Biochemistry, Serotonin. In *StatPearls*. StatPearls Publishing. <http://www.ncbi.nlm.nih.gov/books/NBK560856/>
- Bang, S. J., & Commons, K. G. (2012). Forebrain GABAergic projections from the dorsal raphe nucleus identified by using GAD67–GFP knock-in mice. *Journal of Comparative Neurology*, 520(18), 4157–4167. <https://doi.org/10.1002/cne.23146>
- Bang, S. J., Jensen, P., Dymecki, S. M., & Commons, K. G. (2012). Projections and interconnections of genetically defined serotonin neurons in mice. *European Journal of Neuroscience*, 35(1), 85–96. <https://doi.org/10.1111/j.1460-9568.2011.07936.x>
- Barnes, N. M., Hales, T. G., Lummis, S. C. R., & Peters, J. A. (2009). The 5-HT₃ receptor—The relationship between structure and function. *Neuropharmacology*, 56(1), 273–284. <https://doi.org/10.1016/j.neuropharm.2008.08.003>
- Barnes, N. M., & Sharp, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacology*, 38(8), 1083–1152. [https://doi.org/10.1016/S0028-3908\(99\)00010-6](https://doi.org/10.1016/S0028-3908(99)00010-6)
- Bassett, D. S., & Sporns, O. (2017). Network neuroscience. *Nature Neuroscience*, 20(3), Article 3. <https://doi.org/10.1038/nn.4502>
- Bates, D. M., Mächler, M., Bolker, B. M., Steve Walker, & Walker, S. C. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Beck, S. G., Yu-zhen Pan, Pan, Y.-Z., Akanwa, A., & Kirby, L. G. (2004). Median and dorsal raphe neurons are not electrophysiologically identical. *Journal of Neurophysiology*, 91(2), 994–1005. <https://doi.org/10.1152/jn.00744.2003>

- Bel, N., & Artigas, F. (1992). Fluvoxamine preferentially increases extracellular 5-hydroxytryptamine in the raphe nuclei: An in vivo microdialysis study. *European Journal of Pharmacology*, 229(1), 101–103. [https://doi.org/10.1016/0014-2999\(92\)90292-C](https://doi.org/10.1016/0014-2999(92)90292-C)
- Belin, M.-F., M. Aguera, M. Tappaz, A. McRae-Degueurce, P. Bobillier, & J. F. Pujol. (1979). GABA-accumulating neurons in the nucleus raphe dorsalis and periaqueductal gray in the rat: A biochemical and radioautographic study. *Brain Research*, 170(2), 279–297. [https://doi.org/10.1016/0006-8993\(79\)90107-0](https://doi.org/10.1016/0006-8993(79)90107-0)
- Bengel, D., Jöhren, O., Andrews, A. M., Heils, A., Mößner, R., Mößner, R., Sanvitto, G. L., Saavedra, J. M., Lesch, K.-P., & Murphy, D. L. (1997). Cellular localization and expression of the serotonin transporter in mouse brain. *Brain Research*, 778(2), 338–345. [https://doi.org/10.1016/S0006-8993\(97\)01080-9](https://doi.org/10.1016/S0006-8993(97)01080-9)
- Berényi, A., Zoltán Somogyvári, Somogyvári, Z., Nagy, A., Roux, L., Long, J. D., Fujisawa, S., Stark, E., Leonardo, A., Harris, T. D., & Buzsáki, G. (2014). Large-scale, high-density (up to 512 channels) recording of local circuits in behaving animals. *Journal of Neurophysiology*, 111(5), 1132–1149. <https://doi.org/10.1152/jn.00785.2013>
- Bettencourt, L. M. A., Stephens, G. J., Ham, M. I., & Gross, G. W. (2007). Functional structure of cortical neuronal networks grown in vitro. *Physical Review. E, Statistical, Nonlinear, and Soft Matter Physics*, 75(2 Pt 1), 021915. <https://doi.org/10.1103/PhysRevE.75.021915>
- Blier, P., & Bouchard, C. (1994). Modulation of 5-HT release in the guinea-pig brain following long-term administration of antidepressant drugs. *British Journal*

- of *Pharmacology*, 113(2), 485–495. <https://doi.org/10.1111/j.1476-5381.1994.tb17015.x>
- Blier, P., & de Montigny, C. (1983). Electrophysiological investigations on the effect of repeated zimelidine administration on serotonergic neurotransmission in the rat. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 3(6), 1270–1278.
- Blier, P., & de Montigny, C. (1994). Current advances and trends in the treatment of depression. *Trends in Pharmacological Sciences*, 15(7), 220–226. [https://doi.org/10.1016/0165-6147\(94\)90315-8](https://doi.org/10.1016/0165-6147(94)90315-8)
- Blier, P., & El Mansari, M. (2013). Serotonin and beyond: Therapeutics for major depression. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 368(1615), 20120536. <https://doi.org/10.1098/rstb.2012.0536>
- Bockaert, J., Sylvie Claeyssen, Claeyssen, S., Dumuis, A., & Marin, P. (2010). Classification and Signaling Characteristics of 5-HT Receptors. *Handbook of Behavioral Neuroscience*, 21, 103–121. [https://doi.org/10.1016/s1569-7339\(10\)70073-4](https://doi.org/10.1016/s1569-7339(10)70073-4)
- Boothman, L. J., Raley, J., Denk, F., Hirani, E., & Sharp, T. (2006). In vivo evidence that 5-HT(2C) receptors inhibit 5-HT neuronal activity via a GABAergic mechanism. *British Journal of Pharmacology*, 149(7), 861–869. <https://doi.org/10.1038/sj.bjp.0706935>
- Boothman, L. J., & Sharp, T. (2005). A role for midbrain raphe gamma aminobutyric acid neurons in 5-hydroxytryptamine feedback control. *Neuroreport*, 16(9), 891–896. <https://doi.org/10.1097/00001756-200506210-00004>

- Bortolato, M., Chen, K., & Shih, J. C. (2010). The Degradation of Serotonin: Role of MAO. *Handbook of Behavioral Neuroscience*, 21, 203–218.
[https://doi.org/10.1016/s1569-7339\(10\)70079-5](https://doi.org/10.1016/s1569-7339(10)70079-5)
- Boyle, L., Posani, L., Irfan, S., Siegelbaum, S. A., & Fusi, S. (2022). *The geometry of hippocampal CA2 representations enables abstract coding of social familiarity and identity* (p. 2022.01.24.477361). bioRxiv.
<https://doi.org/10.1101/2022.01.24.477361>
- Bragin, A., J Hetke, Hetke, J. F., Wilson, C. L., Anderson, D. J., Engel, J., Engel, J., & Buzsáki, G. (2000). Multiple site silicon-based probes for chronic recordings in freely moving rats: Implantation, recording and histological verification. *Journal of Neuroscience Methods*, 98(1), 77–82.
[https://doi.org/10.1016/s0165-0270\(00\)00193-x](https://doi.org/10.1016/s0165-0270(00)00193-x)
- Brischoux, F., Chakraborty, S., Brierley, D. I., & Ungless, M. A. (2009). Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. *Proceedings of the National Academy of Sciences*, 106(12), 4894–4899.
<https://doi.org/10.1073/pnas.0811507106>
- Brouard, J. T., Schweimer, J. V., Houlton, R., Burnham, K. E., Quérée, P., & Sharp, T. (2015). Pharmacological Evidence for 5-HT₆ Receptor Modulation of 5-HT Neuron Firing in Vivo. *ACS Chemical Neuroscience*, 6(7), 1241–1247.
<https://doi.org/10.1021/acscchemneuro.5b00061>
- Brown, W. A., & Rosdolsky, M. (2015). The clinical discovery of imipramine. *The American Journal of Psychiatry*, 172(5), 426–429.
<https://doi.org/10.1176/appi.ajp.2015.14101336>

- Burgstaller, J., Hindinger, E., Gesierich, B., & Baier, H. (2019). *Light-sheet imaging and graph-theoretical analysis of antidepressant action in the larval zebrafish brain network*. <https://doi.org/10.1101/618843>
- Buzsáki, G. (2004). Large-scale recording of neuronal ensembles. *Nature Neuroscience*, 7(5), Article 5. <https://doi.org/10.1038/nn1233>
- Calizo, L. H., Akanwa, A., Ma, X., Yu-zhen Pan, Pan, Y. Z., Pan, Y.-Z., Lemos, J. C., Craige, C., Heemstra, L. A., Beck, S. G., & Beck, S. G. (2011). Raphe serotonin neurons are not homogenous: Electrophysiological, morphological and neurochemical evidence. *Neuropharmacology*, 61(3), 524–543. <https://doi.org/10.1016/j.neuropharm.2011.04.008>
- Cardin, J. A. (2018). Inhibitory Interneurons Regulate Temporal Precision and Correlations in Cortical Circuits. *Trends in Neurosciences*, 41(10), 689–700. <https://doi.org/10.1016/j.tins.2018.07.015>
- Carillo-Reid, L., & Yuste, R. (2020). What Is a Neuronal Ensemble? In *Oxford Research Encyclopedia of Neuroscience*.
- Carlson, D. E., & Carin, L. (2019). Continuing progress of spike sorting in the era of big data. *Current Opinion in Neurobiology*, 55, 90–96. <https://doi.org/10.1016/j.conb.2019.02.007>
- Carrillo-Reid, L., Han, S., Yang, W., Akrouh, A., & Yuste, R. (2019). Controlling Visually Guided Behavior by Holographic Recalling of Cortical Ensembles. *Cell*, 178(2), 447–457.e5. <https://doi.org/10.1016/j.cell.2019.05.045>
- Carrillo-Reid, L., Yang, W., Miller, J. K., Peterka, D. S., & Yuste, R. (2017). Imaging and Optically Manipulating Neuronal Ensembles. *Annual Review of Biophysics*, 46(1), 271–293. <https://doi.org/10.1146/annurev-biophys-070816-033647>

- Ceglia, I., Acconcia, S., Fracasso, C., Colovic, M., Caccia, S., & Invernizzi, R. W. (2004). Effects of chronic treatment with escitalopram or citalopram on extracellular 5-HT in the prefrontal cortex of rats: Role of 5-HT_{1A} receptors. *British Journal of Pharmacology*, 142(3), 469–478. <https://doi.org/10.1038/sj.bjp.0705800>
- Challis, C., Boulden, J., Veerakumar, A., Espallergues, J., Vassoler, F. M., Pierce, R. C., Pierce, R. C., Pierce Rc, Beck, S. G., Olivier Berton, Berton, O., & Olivier Berton. (2013). Raphe GABAergic neurons mediate the acquisition of avoidance after social defeat. *The Journal of Neuroscience*, 33(35), 13978–13988. <https://doi.org/10.1523/jneurosci.2383-13.2013>
- Chaput, Y., Blier, P., & de Montigny, C. (1986). In vivo electrophysiological evidence for the regulatory role of autoreceptors on serotonergic terminals. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 6(10), 2796–2801.
- Chaput, Y., de Montigny, C., & Blier, P. (1986). Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: Electrophysiological studies in the rat brain. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 333(4), 342–348. <https://doi.org/10.1007/BF00500007>
- Chaput, Y., de Montigny, C., & Blier, P. (1991). Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments. An in vivo electrophysiologic study in the rat. *Neuropsychopharmacology*, 5(4), 219–229.
- Cho, J. R., Treweek, J. B., Robinson, J. E., Xiao, C., Bremner, L. R., Greenbaum, A., & Gradinaru, V. (2017). Dorsal Raphe Dopamine Neurons Modulate Arousal and

- Promote Wakefulness by Salient Stimuli. *Neuron*, 94(6), 1205.
<https://doi.org/10.1016/j.neuron.2017.05.020>
- Cipriani, A., Furukawa, T. A., Salanti, G., Chaimani, A., Atkinson, L. Z., Ogawa, Y., Leucht, S., Ruhé, H. G., Turner, E. H., Higgins, J. P. T., Egger, M., Takeshima, N., Hayasaka, Y., Imai, H., Shinohara, K., Tajika, A., Ioannidis, J. P. A., & Geddes, J. R. (2018). Comparative Efficacy and Acceptability of 21 Antidepressant Drugs for the Acute Treatment of Adults With Major Depressive Disorder: A Systematic Review and Network Meta-Analysis. *The Lancet*, 391(10128), 1357–1366. [https://doi.org/10.1016/s0140-6736\(17\)32802-7](https://doi.org/10.1016/s0140-6736(17)32802-7)
- Citrome, L. (2011). Olanzapine-fluoxetine combination for the treatment of bipolar depression. *Expert Opinion on Pharmacotherapy*, 12(17), 2751–2758.
<https://doi.org/10.1517/14656566.2011.632368>
- Cohen, J. Y., Amoroso, M. W., & Uchida, N. (2015). Serotonergic neurons signal reward and punishment on multiple timescales. *ELife*, 4, e06346.
<https://doi.org/10.7554/eLife.06346>
- Cohen, J. Y., & Grossman, C. D. (2020). Dorsal raphe serotonergic neurons regulate behavior on multiple timescales. 31, 521–529. <https://doi.org/10.1016/b978-0-444-64125-0.00030-x>
- Cohen, & Kohn, A. (2011). Measuring and interpreting neuronal correlations. *Nature Neuroscience*, 14(7), 811–819. <https://doi.org/10.1038/nn.2842>
- Commons, K. G. (2015). Two major network domains in the dorsal raphe nucleus. *The Journal of Comparative Neurology*, 523(10), 1488–1504.
<https://doi.org/10.1002/cne.23748>

- Cools, R., Roberts, A. C., & Robbins, T. W. (2008). Serotonergic regulation of emotional and behavioural control processes. *Trends in Cognitive Sciences*, 12(1), 31–40. <https://doi.org/10.1016/j.tics.2007.10.011>
- Coppen, A. (1967). The biochemistry of affective disorders. *The British Journal of Psychiatry: The Journal of Mental Science*, 113(504), 1237–1264. <https://doi.org/10.1192/bjp.113.504.1237>
- Cornea-Hébert, V., Riad, M., Wu, C., Singh, S. K., & Descarries, L. (1999). Cellular and subcellular distribution of the serotonin 5-HT_{2A} receptor in the central nervous system of adult rat. *The Journal of Comparative Neurology*, 409(2), 187–209. [https://doi.org/10.1002/\(sici\)1096-9861\(19990628\)409:2<187::aid-cne2>3.0.co;2-p](https://doi.org/10.1002/(sici)1096-9861(19990628)409:2<187::aid-cne2>3.0.co;2-p)
- Cowen, P. J., & Browning, M. (2015). What has serotonin to do with depression? *World Psychiatry*, 14(2), 158–160. <https://doi.org/10.1002/wps.20229>
- Czachura, J. F., & Rasmussen, K. (2000). Effects of acute and chronic administration of fluoxetine on the activity of serotonergic neurons in the dorsal raphe nucleus of the rat. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 362(3), 266–275. <https://doi.org/10.1007/s002100000290>
- Dahlström, A., Annica Dahlström, Dahlström, A., & Fuxe, K. (1964). Localization of monoamines in the lower brain stem. *Cellular and Molecular Life Sciences*, 20(7), 398–399. <https://doi.org/10.1007/bf02147990>
- Dankoski, E. C., Carroll, S., & Wightman, R. M. (2016). Acute selective serotonin reuptake inhibitors regulate the dorsal raphe nucleus causing amplification of terminal serotonin release. *Journal of Neurochemistry*, 136(6), 1131–1141. <https://doi.org/10.1111/jnc.13528>

- Daw, N. D., Kakade, S., & Dayan, P. (2002). Opponent interactions between serotonin and dopamine. *Neural Networks: The Official Journal of the International Neural Network Society*, 15(4–6), 603–616. [https://doi.org/10.1016/s0893-6080\(02\)00052-7](https://doi.org/10.1016/s0893-6080(02)00052-7)
- Dayan, P., & Huys, Q. J. M. (2009). Serotonin in affective control. *Annual Review of Neuroscience*, 32, 95–126. <https://doi.org/10.1146/annurev.neuro.051508.135607>
- Dayan, P., Huys, Q. J. M., Mw, A., & Uchida, N. (2015). *Serotonin's many meanings elude simple theories Neurons that produce serotonin respond in a number of different and complex ways in anticipation and receipt of rewards or punishments.*
- de la Rocha, J., Doiron, B., Shea-Brown, E., Josić, K., & Reyes, A. D. (2007). Correlation between neural spike trains increases with firing rate. *Nature*, 448(7155), 802–806. <https://doi.org/10.1038/nature06028>
- de Vries, S. E. J., Lecoq, J., Buice, M. A., Groblewski, P. A., Ocker, G. K., Oliver, M., Oliver, M., Feng, D., Cain, N., Ledochowitsch, P., Millman, D., Roll, K., Garrett, M., Tom Keenan, Keenan, T., Tom Keenan, Kuan, L., Mihalas, S., Olsen, S. R., ... Koch, C. (2020). A large-scale standardized physiological survey reveals functional organization of the mouse visual cortex. *Nature Neuroscience*, 23(1), 138–151. <https://doi.org/10.1038/s41593-019-0550-9>
- De Wilde, J., de Wilde, J., Spiers, R., Mertens, C., Mertens, C., Bartholomé, F., Schotte, G., & Leyman, S. (1993). A double-blind, comparative, multicentre study comparing paroxetine with fluoxetine in depressed patients. *Acta Psychiatrica Scandinavica*, 87(2), 141–145. <https://doi.org/10.1111/j.1600-0447.1993.tb03345.x>

- Deakin, J. F., & Graeff, F. G. (1991). 5-HT and mechanisms of defence. *Journal of Psychopharmacology* (Oxford, England), 5(4), 305–315. <https://doi.org/10.1177/026988119100500414>
- Deguchi, T., Deguchi, T., & Barchas, J. D. (1972). Regional distribution and developmental change of tryptophan hydroxylase activity in rat brain. *Journal of Neurochemistry*, 19(3), 927–929. <https://doi.org/10.1111/j.1471-4159.1972.tb01412.x>
- Descarries, L., & Riad, M. (2012). Effects of the antidepressant fluoxetine on the subcellular localization of 5-HT_{1A} receptors and SERT. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1601), 2416–2425. <https://doi.org/10.1098/rstb.2011.0361>
- Descarries, L., Riad, M., & Parent, M. (2010). Ultrastructure of the Serotonin Innervation in the Mammalian Central Nervous System. *Handbook of Behavioral Neuroscience*, 21, 65–101. [https://doi.org/10.1016/s1569-7339\(10\)70072-2](https://doi.org/10.1016/s1569-7339(10)70072-2)
- Descarries, L., Watkins, K. C., Garcia, S., & Beaudet, A. (1982). The serotonin neurons in nucleus raphe dorsalis of adult rat: A light and electron microscope radioautographic study. *The Journal of Comparative Neurology*, 207(3), 239–254. <https://doi.org/10.1002/cne.902070305>
- DeVane, C. L., & Cl, D. (1999). Metabolism and pharmacokinetics of selective serotonin reuptake inhibitors. *Cellular and Molecular Neurobiology*, 19(4), 443–466. <https://doi.org/10.1023/a:1006934807375>
- Diba, K., Amarasingham, A., Mizuseki, K., & Buzsáki, G. (2014). Millisecond Timescale Synchrony among Hippocampal Neurons. *The Journal of*

- Neuroscience*, 34(45), 14984–14994. <https://doi.org/10.1523/jneurosci.1091-14.2014>
- Dickenson, A. H., Oliveras, J.-L., & Besson, J.-M. (1979). Role of the nucleus raphe magnus in opiate analgesia as studied by the microinjection technique in the rat. *Brain Research*, 170(1), 95–111. [https://doi.org/10.1016/0006-8993\(79\)90943-0](https://doi.org/10.1016/0006-8993(79)90943-0)
- Dolzani, S. D., Baratta, M., Baratta, M. V., Amat, J., Kara L. Agster, Agster, K. L., Saddoris, M. P., Watkins, L. R., & Maier, S. F. (2016). Activation of a Habenulo–Raphe Circuit Is Critical for the Behavioral and Neurochemical Consequences of Uncontrollable Stress in the Male Rat. *ENeuro*, 3(5). <https://doi.org/10.1523/eneuro.0229-16.2016>
- Dong, J., de Montigny, C., & Blier, P. (1999). Assessment of the serotonin reuptake blocking property of YM992: Electrophysiological studies in the rat hippocampus and dorsal raphe. *Synapse (New York, N.Y.)*, 34(4), 277–289. [https://doi.org/10.1002/\(SICI\)1098-2396\(19991215\)34:4<277::AID-SYN4>3.0.CO;2-W](https://doi.org/10.1002/(SICI)1098-2396(19991215)34:4<277::AID-SYN4>3.0.CO;2-W)
- Dorocic, I. P., Fürth, D., Xuan, Y., Johansson, Y., Pozzi, L., Silberberg, G., Carlén, M., & Meletis, K. (2014). A Whole-Brain Atlas of Inputs to Serotonergic Neurons of the Dorsal and Median Raphe Nuclei. *Neuron*, 83(3), 663–678. <https://doi.org/10.1016/j.neuron.2014.07.002>
- Dowben, R. M., Jerzy E. Rose, & Rose, J. E. (1953). A metal-filled microelectrode. *Science*, 118(3053), 22–24. <https://doi.org/10.1126/science.118.3053.22>
- Ecker, A. S., Berens, P., Cotton, R. J., Cotton, R., Subramaniyan, M., Denfield, G. H., Cadwell, C. R., Smirnakis, S. M., Bethge, M., & Tolias, A. S. (2014). State

- dependence of noise correlations in macaque primary visual cortex. *Neuron*, 82(1), 235–248. <https://doi.org/10.1016/j.neuron.2014.02.006>
- English, D. F., McKenzie, S., Evans, T., Kim, K., Yoon, E., & Buzsáki, G. (2017). Pyramidal Cell-Interneuron Circuit Architecture and Dynamics in Hippocampal Networks. *Neuron*, 96(2), 505–520.e7. <https://doi.org/10.1016/j.neuron.2017.09.033>
- Erickson, J. D., Schafer, M. K., Bonner, T. I., Eiden, L. E., & Weihe, E. (1996). Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. *Proceedings of the National Academy of Sciences*, 93(10), 5166–5171. <https://doi.org/10.1073/pnas.93.10.5166>
- Ersparmer, V., & Asero, B. (1952). Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature*, 169(4306). <https://doi.org/10.1038/169800b0>
- Fallon, J. H., & Loughlin, S. E. (1982). Monoamine innervation of the forebrain: Collateralization. *Brain Research Bulletin*, 9(1), 295–307. [https://doi.org/10.1016/0361-9230\(82\)90143-5](https://doi.org/10.1016/0361-9230(82)90143-5)
- Fernandez, S. P., Bruno Cauli, Cauli, B., Cabezaz, C., Muzerelle, A., Poncer, J. C., & Gaspar, P. (2016). Multiscale single-cell analysis reveals unique phenotypes of raphe 5-HT neurons projecting to the forebrain. *Brain Structure & Function*, 221(8), 4007–4025. <https://doi.org/10.1007/s00429-015-1142-4>
- Fiáth, R., Meszéna, D., Somogyvári, Z., Boda, M., Bartho, P., Ruther, P., István Ulbert, István Ulbert, & Ulbert, I. (2021). Recording site placement on planar silicon-based probes affects signal quality in acute neuronal recordings. *Scientific Reports*, 11(1), 2028–2028. <https://doi.org/10.1038/s41598-021-81127-5>

- Fink, K., & Göthert, M. (2007). 5-HT Receptor Regulation of Neurotransmitter Release. *Pharmacological Reviews*, 59(4), 360–417.
<https://doi.org/10.1124/pr.59.07103>
- Fitzpatrick, P. F. (1999). Tetrahydropterin-dependent amino acid hydroxylases. *Annual Review of Biochemistry*, 68(1), 355–381.
<https://doi.org/10.1146/annurev.biochem.68.1.355>
- Fornal, C. A., Metzler, C. W., Marrosu, F., Ribiero-do-Valle, L. E., & Jacobs, B. L. (1996). A subgroup of dorsal raphe serotonergic neurons in the cat is strongly activated during oral-buccal movements. *Brain Research*, 716(1–2), 123–133.
[https://doi.org/10.1016/0006-8993\(96\)00006-6](https://doi.org/10.1016/0006-8993(96)00006-6)
- Fritze, S., Spanagel, R., & Noori, H. R. (2017). Adaptive dynamics of the 5-HT systems following chronic administration of selective serotonin reuptake inhibitors: A meta-analysis. *Journal of Neurochemistry*, 142(5), 747–755.
<https://doi.org/10.1111/jnc.14114>
- Fujita, M., Masahiro Fujita, Shimada, S., Maeno, H., Maeno, H., Nishimura, T., Nishimura, T., & Tohyama, M. (1993). Cellular localization of serotonin transporter mRNA in the rat brain. *Neuroscience Letters*, 162, 59–62.
[https://doi.org/10.1016/0304-3940\(93\)90559-4](https://doi.org/10.1016/0304-3940(93)90559-4)
- Fusi, S., Miller, E. K., & Rigotti, M. (2016). Why neurons mix: High dimensionality for higher cognition. *Current Opinion in Neurobiology*, 37, 66–74.
<https://doi.org/10.1016/j.conb.2016.01.010>
- Fustiñana, M. S., Eichlisberger, T., Bouwmeester, T., Bitterman, Y., & Lüthi, A. (2021). State-dependent encoding of exploratory behaviour in the amygdala. *Nature*, 592(7853), 267–271. <https://doi.org/10.1038/s41586-021-03301-z>

- Fuxe, K., Dahlström, A., Jonsson, G., Marcellino, D., Guescini, M., Dam, M., Manger, P. R., & Agnati, L. F. (2010). The discovery of central monoamine neurons gave volume transmission to the wired brain. *Progress in Neurobiology*, 90(2), 82–100. <https://doi.org/10.1016/j.pneurobio.2009.10.012>
- G. Gartlehner, R. Hansen, L. Morgan, K. Thaler, L. Lux, M. Noord, U. Mager, P. Thieda, B. Gaynes, Tania M. Wilkins, M. Strobelberger, S. Lloyd, U. Reichenpfader, & K. Lohr. (2011). *Comparative Benefits and Harms of Second-Generation Antidepressants for Treating Major Depressive Disorder An Updated Meta-analysis*.
- Gagnon, D., & Parent, M. (2014). Distribution of VGLUT3 in Highly Collateralized Axons from the Rat Dorsal Raphe Nucleus as Revealed by Single-Neuron Reconstructions. *PLOS ONE*, 9(2), e87709. <https://doi.org/10.1371/journal.pone.0087709>
- Gamrani, H., Calas, A., Belin, M. F., Aguera, M., & Pujol, J. F. (1979). High resolution radioautographic identification of [3H]GABA labeled neurons in the rat nucleus raphe dorsalis. *Neuroscience Letters*, 15(1), 43–48. [https://doi.org/10.1016/0304-3940\(79\)91527-1](https://doi.org/10.1016/0304-3940(79)91527-1)
- Gartlehner, G., Hansen, R. A., Morgan, L. C., Thaler, K. J., Thaler, K., Lux, L. J., Van Noord, M., Mager, U., Thieda, P., Gaynes, B. N., Bradley N. Gaynes, Wilkins, T. M., Strobelberger, M., Lloyd, S., Reichenpfader, U., & Lohr, K. N. (2011). Comparative Benefits and Harms of Second-Generation Antidepressants for Treating Major Depressive Disorder. *Annals of Internal Medicine*. <https://doi.org/10.7326/0003-4819-155-11-201112060-00009>
- Gartside, S. E., Umbers, V., Hajós, M., & Sharp, T. (1995). Interaction between a selective 5-HT_{1A} receptor antagonist and an SSRI in vivo: Effects on 5-HT cell

- firing and extracellular 5-HT. *British Journal of Pharmacology*, 115(6), 1064–1070. <https://doi.org/10.1111/j.1476-5381.1995.tb15919.x>
- Gaspar, P., & Lillesaar, C. (2012). Probing the diversity of serotonin neurons. *Philosophical Transactions of the Royal Society B*, 367(1601), 2382–2394. <https://doi.org/10.1098/rstb.2011.0378>
- Georgopoulos, A. P., Schwartz, A. B., & Kettner, R. (1986). Neuronal Population Coding of Movement Direction. *Science*, 233(4771), 1416–1419. <https://doi.org/10.1126/science.3749885>
- Gerhard, F., Pipa, G., Lima, B., Neuenschwander, S., & Gerstner, W. (2011). Extraction of Network Topology From Multi-Electrode Recordings: Is there a Small-World Effect? *Frontiers in Computational Neuroscience*, 5, 4. <https://doi.org/10.3389/fncom.2011.00004>
- Gervasoni, D., Peyron, C., Rampon, C., Barbagli, B., Chouvet, G., Urbain, N., Fort, P., & Luppi, P. H. (2000). Role and origin of the GABAergic innervation of dorsal raphe serotonergic neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 20(11), 4217–4225.
- Gold, C., Henze, D. A., Koch, C., & Buzsáki, G. (2006). On the Origin of the Extracellular Action Potential Waveform: A Modeling Study. *Journal of Neurophysiology*, 95(5), 3113–3128. <https://doi.org/10.1152/jn.00979.2005>
- Graeff, F. G., Guimarães, F. S., De Andrade, T. G., & Deakin, J. F. (1996). Role of 5-HT in stress, anxiety, and depression. *Pharmacology, Biochemistry, and Behavior*, 54(1), 129–141. [https://doi.org/10.1016/0091-3057\(95\)02135-3](https://doi.org/10.1016/0091-3057(95)02135-3)
- Grahn, R. E., Will, M. J., Hammack, S. E., Maswood, S., McQueen, M. B., Watkins, L. R., & Maier, S. F. (1999). Activation of serotonin-immunoreactive cells in the

- dorsal raphe nucleus in rats exposed to an uncontrollable stressor. *Brain Research*, 826(1), 35–43. [https://doi.org/10.1016/S0006-8993\(99\)01208-1](https://doi.org/10.1016/S0006-8993(99)01208-1)
- Grimsby, J., & Wang, L.-J. (1991). Human monoamine oxidase A and B genes exhibit identical exon-intron organization. *Proceedings of the National Academy of Sciences of the United States of America*. <https://doi.org/10.1073/pnas.88.9.3637>
- Grzanna, R., & Molliver, M. E. (1980). The locus coeruleus in the rat: An immunohistochemical delineation. *Neuroscience*, 5(1), 21–40. [https://doi.org/10.1016/0306-4522\(80\)90068-8](https://doi.org/10.1016/0306-4522(80)90068-8)
- Guolin Ke, Ke, G., Meng, Q., Finley, T., Wang, T., Wei Chen, Wei Chen, Chen, W., Chen, W., Ma, W., Ye, Q., & Liu, T.-Y. (2017). LightGBM: a highly efficient gradient boosting decision tree. *NIPS*, 30, 3149–3157.
- Gutierrez, R., Carmenta, J. M., Nicolelis, M. A. L., & Simon, S. A. (2006). Orbitofrontal Ensemble Activity Monitors Licking and Distinguishes Among Natural Rewards. *Journal of Neurophysiology*, 95(1), 119–133. <https://doi.org/10.1152/jn.00467.2005>
- Haddjeri, N., Lavoie, N., & Blier, P. (2004). Electrophysiological evidence for the tonic activation of 5-HT(1A) autoreceptors in the rat dorsal raphe nucleus. *Neuropsychopharmacology*, 29(10), 1800–1806. <https://doi.org/10.1038/sj.npp.1300489>
- Hagberg, A., Schult, D. A., & Swart, P. J. (2008). *Exploring Network Structure, Dynamics, and Function using NetworkX*.
- Hajós, M., Allers, K. A., Jennings, K., Sharp, T., Charette, G., Sík, A., & Kocsis, B. (2007). Neurochemical identification of stereotypic burst-firing neurons in the rat dorsal raphe nucleus using juxtacellular labelling methods. *The European*

- Journal of Neuroscience*, 25(1), 119–126. <https://doi.org/10.1111/j.1460-9568.2006.05276.x>
- Hajós, M., Richards, C. D., Richards, C. D., Székely, A. D., & Sharp, T. (1998). An electrophysiological and neuroanatomical study of the medial prefrontal cortical projection to the midbrain raphe nuclei in the rat. *Neuroscience*, 87(1), 95–108. [https://doi.org/10.1016/s0306-4522\(98\)00157-2](https://doi.org/10.1016/s0306-4522(98)00157-2)
- Halberstadt, A. L., & Balaban, C. D. (2006). Serotonergic and nonserotonergic neurons in the dorsal raphe nucleus send collateralized projections to both the vestibular nuclei and the central amygdaloid nucleus. *Neuroscience*, 140(3), 1067–1077. <https://doi.org/10.1016/j.neuroscience.2006.02.053>
- Hale, M. W., & Lowry, C. A. (2011). Functional topography of midbrain and pontine serotonergic systems: Implications for synaptic regulation of serotonergic circuits. *Psychopharmacology*, 213(2–3), 243–264. <https://doi.org/10.1007/s00213-010-2089-z>
- Hardcastle, K., Maheswaranathan, N., Ganguli, S., & Giocomo, L. M. (2017). A Multiplexed, Heterogeneous, and Adaptive Code for Navigation in Medial Entorhinal Cortex. *Neuron*, 94(2), 375–387.e7. <https://doi.org/10.1016/j.neuron.2017.03.025>
- Hayashi, K., Nakao, K., & Nakamura, K. (2015). Appetitive and Aversive Information Coding in the Primate Dorsal Raphé Nucleus. *The Journal of Neuroscience*, 35(15), 6195–6208. <https://doi.org/10.1523/jneurosci.2860-14.2015>
- Hebb, D. O. (1949). *The organization of behavior; a neuropsychological theory* (pp. xix, 335). Wiley.

- Hensler, J. G. (2003). Regulation of 5-HT_{1A} receptor function in brain following agonist or antidepressant administration. *Life Sciences*, 72(15), 1665–1682. [https://doi.org/10.1016/S0024-3205\(02\)02482-7](https://doi.org/10.1016/S0024-3205(02)02482-7)
- Heym, J., Trulson, M. E., & Jacobs, B. L. (1982). Raphe unit activity in freely moving cats: Effects of phasic auditory and visual stimuli. *Brain Research*, 232(1), 29–39. [https://doi.org/10.1016/0006-8993\(82\)90608-4](https://doi.org/10.1016/0006-8993(82)90608-4)
- Hill, D., Mehta, S. B., & Kleinfeld, D. (2011). Quality metrics to accompany spike sorting of extracellular signals. *The Journal of Neuroscience*, 31(24), 8699–8705. <https://doi.org/10.1523/jneurosci.0971-11.2011>
- Hirschfeld, R. M. (2000). History and evolution of the monoamine hypothesis of depression. *The Journal of Clinical Psychiatry*, 61 Suppl 6, 4–6.
- Hjorth, S. (2016). Looking back (and in)to the future: A personal reflection on ‘Serotonin autoreceptor function and antidepressant drug action’ (Hjorth et al., 2000). *Journal of Psychopharmacology*, 30(11), 1129–1136. <https://doi.org/10.1177/0269881116647621>
- Hjorth, S., Bengtsson, H. J., Kullberg, A., Carlzon, D., Peilot, H., & Auerbach, S. B. (2000). Serotonin autoreceptor function and antidepressant drug action. *Journal of Psychopharmacology*, 14(2), 177–185. <https://doi.org/10.1177/026988110001400208>
- Hofer, S. B., Ko, H., Pichler, B., Vogelstein, J., Ros, H., Zeng, H., Lein, E., Lesica, N. A., & Mrsic-Flogel, T. D. (2011). Differential connectivity and response dynamics of excitatory and inhibitory neurons in visual cortex. *Nature Neuroscience*, 14(8), 1045–1052. <https://doi.org/10.1038/nn.2876>
- Holt, G. R., Softky, W. R., Koch, C., & Douglas, R. J. (1996). Comparison of discharge variability in vitro and in vivo in cat visual cortex neurons. *Journal of*

- Neurophysiology*, 75(5), 1806–1814.
<https://doi.org/10.1152/jn.1996.75.5.1806>
- Huang, K. W., Ochandarena, N. E., Philson, A. C., Hyun, M., Birnbaum, J. E., Cicconet, M., & Sabatini, B. L. (2019). Molecular and anatomical organization of the dorsal raphe nucleus. *ELife*, 8, e46464.
<https://doi.org/10.7554/eLife.46464>
- Hubel, D. H., & Hubel, D. H. (1957). Tungsten Microelectrode for Recording from Single Units. *Science*, 125(3247), 549–550.
<https://doi.org/10.1126/science.125.3247.549>
- Huda, R., Sipe, G. O., Breton-Provencher, V., Cruz, K. G., Pho, G. N., Pho, G. N., Adam, E. M., Gunter, L. M., Sullins, A., Wickersham, I. R., & Sur, M. (2020). Distinct prefrontal top-down circuits differentially modulate sensorimotor behavior. *Nature Communications*, 11(1), 6007–6007.
<https://doi.org/10.1038/s41467-020-19772-z>
- Humphries, M. D. (2011). Spike-Train Communities: Finding Groups of Similar Spike Trains. *Journal of Neuroscience*, 31(6), 2321–2336.
<https://doi.org/10.1523/JNEUROSCI.2853-10.2011>
- Humphries, M. D. (2018). Dynamical networks: Finding, measuring, and tracking neural population activity using network science. *Network Neuroscience (Cambridge, Mass.)*, 1(4), 324–338.
https://doi.org/10.1162/NETN_a_00020
- Hung, C. P., Kreiman, G., Poggio, T., & DiCarlo, J. J. (2005). Fast Readout of Object Identity from Macaque Inferior Temporal Cortex. *Science*, 310(5749), 863–866. <https://doi.org/10.1126/science.1117593>

- Hyttel, J., & Hyttel, J. (1994). Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs). *International Clinical Psychopharmacology*, 9, 19–26. <https://doi.org/10.1097/00004850-199403001-00004>
- Imai, H., Steindler, D. A., & Kitai, S. T. (1986). The organization of divergent axonal projections from the midbrain raphe nuclei in the rat. *The Journal of Comparative Neurology*, 243(3), 363–380. <https://doi.org/10.1002/cne.902430307>
- Invernizzi, R., Bramante, M., & Samanin, R. (1994). Chronic treatment with citalopram facilitates the effect of a challenge dose on cortical serotonin output: Role of presynaptic 5-HT_{1A} receptors. *European Journal of Pharmacology*, 260(2), 243–246. [https://doi.org/10.1016/0014-2999\(94\)90344-1](https://doi.org/10.1016/0014-2999(94)90344-1)
- Ishimura, K., Takeuchi, Y., Fujiwara, K., Tominaga, M., Yoshioka, H., & Sawada, T. (1988). Quantitative analysis of the distribution of serotonin-immunoreactive cell bodies in the mouse brain. *Neuroscience Letters*, 91(3), 265–270. [https://doi.org/10.1016/0304-3940\(88\)90691-X](https://doi.org/10.1016/0304-3940(88)90691-X)
- Jacobs, B. L., & Azmitia, E. C. (1992). Structure and function of the brain serotonin system. *Physiological Reviews*, 72(1), 165–229. <https://doi.org/10.1152/physrev.1992.72.1.165>
- Jacobs, B. L., & Fornal, C. A. (1997). Serotonin and motor activity. *Current Opinion in Neurobiology*, 7(6), 820–825. [https://doi.org/10.1016/S0959-4388\(97\)80141-9](https://doi.org/10.1016/S0959-4388(97)80141-9)
- Jacobs, B. L., & Fornal, C. A. (1999). Activity of serotonergic neurons in behaving animals. *Neuropsychopharmacology: Official Publication of the American*

- College of Neuropsychopharmacology*, 21(2 Suppl), 9S-15S.
[https://doi.org/10.1016/S0893-133X\(99\)00012-3](https://doi.org/10.1016/S0893-133X(99)00012-3)
- Jankowski, M. P., & Sesack, S. R. (2004). Prefrontal cortical projections to the rat dorsal raphe nucleus: Ultrastructural features and associations with serotonin and γ -aminobutyric acid neurons. *The Journal of Comparative Neurology*, 468(4), 518–529. <https://doi.org/10.1002/cne.10976>
- Judge, S. J., Ingram, C. D., & Gartside, S. E. (2004). GABA receptor modulation of 5-HT neuronal firing: Characterization and effect of moderate in vivo variations in glucocorticoid levels. *Neurochemistry International*, 45(7), 1057–1065. <https://doi.org/10.1016/j.neuint.2004.05.003>
- Jun, J. J., Steinmetz, N. A., Siegle, J. H., Denman, D. J., Bauza, M., Barbarits, B., Lee, A. K., Anastassiou, C. A., Andrei, A., Aydin, C., Barbic, M., Blanche, T. J., Bonin, V., Couto, J., Barundeb Dutta, Dutta, B., Gratiy, S. L., Gutnisky, D. A., Häusser, M., ... Harris, T. D. (2017). Fully integrated silicon probes for high-density recording of neural activity. *Nature*, 551(7679), 232–236. <https://doi.org/10.1038/nature24636>
- Kaneko, T., Akiyama, H., Nagatsu, I., & Mizuno, N. (1990). Immunohistochemical demonstration of glutaminase in catecholaminergic and serotonergic neurons of rat brain. *Brain Research*, 507(1), 151–154. [https://doi.org/10.1016/0006-8993\(90\)90535-j](https://doi.org/10.1016/0006-8993(90)90535-j)
- Karnani, M. M., Jackson, J., Ayzenshtat, I., Tucciarone, J., Manoocheri, K., Snider, W. G., & Yuste, R. (2016). Cooperative Subnetworks of Molecularly Similar Interneurons in Mouse Neocortex. *Neuron*, 90(1), 86–100. <https://doi.org/10.1016/j.neuron.2016.02.037>

- Katz, D. B., Simon, S. A., & Nicolelis, M. A. L. (2002). Taste-Specific Neuronal Ensembles in the Gustatory Cortex of Awake Rats. *The Journal of Neuroscience*, 22(5), 1850–1857. <https://doi.org/10.1523/JNEUROSCI.22-05-01850.2002>
- Kaufman, M. T., Benna, M. K., Rigotti, M., Stefanini, F., Fusi, S., & Churchland, A. K. (2022). The implications of categorical and category-free mixed selectivity on representational geometries. *Current Opinion in Neurobiology*, 77, 102644. <https://doi.org/10.1016/j.conb.2022.102644>
- Kawahara, H., Hiroshi Kawahara, Yoshida, M., Yokoo, H., Nishi, M., & Tanaka, M. (1993). Psychological stress increases serotonin release in the rat amygdala and prefrontal cortex assessed by in vivo microdialysis. *Neuroscience Letters*, 162(1), 81–84. [https://doi.org/10.1016/0304-3940\(93\)90565-3](https://doi.org/10.1016/0304-3940(93)90565-3)
- Kendrick & Collinson. (2022). Antidepressants and the serotonin hypothesis of depression. *BMJ*, 01993–01993. <https://doi.org/10.1136/bmj.01993>
- Kirby, L. G., Allen, A. R., & Lucki, I. (1995). Regional differences in the effects of forced swimming on extracellular levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. *Brain Research*, 682(1), 189–196. [https://doi.org/10.1016/0006-8993\(95\)00349-U](https://doi.org/10.1016/0006-8993(95)00349-U)
- Kirby, L. G., Chou-Green, J. M., Davis, K., & Lucki, I. (1997). The effects of different stressors on extracellular 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. *Brain Research*, 760(1–2), 218–230. [https://doi.org/10.1016/S0006-8993\(97\)00287-4](https://doi.org/10.1016/S0006-8993(97)00287-4)
- Kirby, L. G., Luise Pernar, Pernar, L. I. M., Valentino, R. J., & Beck, S. G. (2003). Distinguishing characteristics of serotonin and non-serotonin-containing cells in the dorsal raphe nucleus: Electrophysiological and immunohistochemical

- studies. *Neuroscience*, 116(3), 669–683. [https://doi.org/10.1016/s0306-4522\(02\)00584-5](https://doi.org/10.1016/s0306-4522(02)00584-5)
- Kirby, L. G., Pan, Y.-Z., Freeman-Daniels, E., Rani, S., Nunan, J. D., Akanwa, A., & Beck, S. G. (2007). Cellular effects of swim stress in the dorsal raphe nucleus. *Psychoneuroendocrinology*, 32(6), 712–723. <https://doi.org/10.1016/j.psyneuen.2007.05.001>
- Kocsis, B., Varga, V., Dahan, L., & Sik, A. (2006). Serotonergic neuron diversity: Identification of raphe neurons with discharges time-locked to the hippocampal theta rhythm. *Proceedings of the National Academy of Sciences of the United States of America*, 103(4), 1059–1064. <https://doi.org/10.1073/pnas.0508360103>
- Kodama, N. X., Feng, T., Ullett, J. J., Chiel, H. J., Sivakumar, S. S., & Galán, R. F. (2018). Anti-correlated cortical networks arise from spontaneous neuronal dynamics at slow timescales. *Scientific Reports*, 8(1), 666–666. <https://doi.org/10.1038/s41598-017-18097-0>
- Kohn, A., Coen-Cagli, R., Kanitscheider, I., & Pouget, A. (2016). Correlations and Neuronal Population Information. *Annual Review of Neuroscience*, 39(1), 237–256. <https://doi.org/10.1146/annurev-neuro-070815-013851>
- Kohn, A., & Smith, M. A. (2005). Stimulus Dependence of Neuronal Correlation in Primary Visual Cortex of the Macaque. *The Journal of Neuroscience*, 25(14), 3661–3673. <https://doi.org/10.1523/JNEUROSCI.5106-04.2005>
- Koubi, D., Bezin, L., Cottet-Emard, J.-M., Gharib, A., Bobillier, P., & Sarda, N. (2001). Regulation of expression and enzymatic activities of tyrosine and tryptophan hydroxylases in rat brain after acute electroconvulsive shock. *Brain Research*, 905(1), 161–170. [https://doi.org/10.1016/s0006-8993\(01\)02524-0](https://doi.org/10.1016/s0006-8993(01)02524-0)

- Koyama, Y., Kondo, M., & Shimada, S. (2017). Building a 5-HT_{3A} Receptor Expression Map in the Mouse Brain. *Scientific Reports*, 7(1), 42884–42884. <https://doi.org/10.1038/srep42884>
- Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82(13), 1–26. <https://doi.org/10.18637/jss.v082.i13>
- Laubach, M., Wessberg, J., Wessberg, J., & Nicolelis, M. A. L. (2000). Cortical ensemble activity increasingly predicts behaviour outcomes during learning of a motor task. *Nature*, 405(6786), 567–571. <https://doi.org/10.1038/35014604>
- Lazaridis, I., Tzortzi, O., Weglage, M., Martin, A., Xuan, Y., Marc Parent, Parent, M. A., Johansson, Y., Fuzik, J., Fürth, D., Fenno, L. E., Ramakrishnan, C., Silberberg, G., Deisseroth, K., Carlén, M., & Meletis, K. (2019). A hypothalamus-habenula circuit controls aversion. *Molecular Psychiatry*, 24(9), 1351–1368. <https://doi.org/10.1038/s41380-019-0369-5>
- Lecca, S., Meye, F. J., Meye, F. J., Mameli, M., & Mameli, M. (2014). The lateral habenula in addiction and depression: An anatomical, synaptic and behavioral overview. *European Journal of Neuroscience*, 39(7), 1170–1178. <https://doi.org/10.1111/ejn.12480>
- Lenth, R. V. (2022). *emmeans: Estimated Marginal Means, aka Least-Squares Means*. <https://CRAN.R-project.org/package=emmeans>
- Lesch, K.-P., & Waider, J. (2012). Serotonin in the Modulation of Neural Plasticity and Networks: Implications for Neurodevelopmental Disorders. *Neuron*, 76(1), 175–191. <https://doi.org/10.1016/j.neuron.2012.09.013>

- Levitt, P., Pinar, J. E., & Breakefield, X. O. (1982). Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. *Proceedings of the National Academy of Sciences of the United States of America*, 79(20), 6385–6389. <https://doi.org/10.1073/pnas.79.20.6385>
- Li, Y., Zhong, W., Wang, D., Feng, Q., Liu, Z., Zhou, J., Jia, C., Hu, F., Zeng, J., Guo, Q., Fu, L., & Luo, M. (2016a). Serotonin neurons in the dorsal raphe nucleus encode reward signals. *Nature Communications*, 7(1), Article 1. <https://doi.org/10.1038/ncomms10503>
- Li, Y., Zhong, W., Wang, D., Feng, Q., Liu, Z., Zhou, J., Jia, C., Hu, F., Zeng, J., Guo, Q., Fu, L., & Luo, M. (2016b). Serotonin neurons in the dorsal raphe nucleus encode reward signals. *Nature Communications*, 7(1), 10503–10503. <https://doi.org/10.1038/ncomms10503>
- Liu, R., Jolas, T., & Aghajanian, G. K. (2000). Serotonin 5-HT(2) receptors activate local GABA inhibitory inputs to serotonergic neurons of the dorsal raphe nucleus. *Brain Research*, 873(1), 34–45. [https://doi.org/10.1016/S0006-8993\(00\)02468-9](https://doi.org/10.1016/S0006-8993(00)02468-9)
- Liu, Z., Zhou, J., Li, Y., Hu, F., Lu, Y., Ma, M., Feng, Q., Zhang, J., Wang, D., Zeng, J., Bao, J., Ji-Young Kim, Kim, J.-Y., Kim, J., Chen, Z.-F., Mestikawy, S. E., & Luo, M. (2014). Dorsal Raphe Neurons Signal Reward through 5-HT and Glutamate. *Neuron*, 81(6), 1360–1374. <https://doi.org/10.1016/j.neuron.2014.02.010>
- López-Muñoz, F., & Alamo, C. (2009). Monoaminergic neurotransmission: The history of the discovery of antidepressants from 1950s until today. *Current*

- Pharmaceutical Design*, 15(14), 1563–1586.
<https://doi.org/10.2174/138161209788168001>
- Lu, J., Jhou, T. C., Jhou, T. C., & Saper, C. B. (2006). Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray matter. *The Journal of Neuroscience*, 26(1), 193–202. <https://doi.org/10.1523/jneurosci.2244-05.2006>
- Lucas, G., & Debonnel, G. (2002). 5-HT₄ receptors exert a frequency-related facilitatory control on dorsal raphe nucleus 5-HT neuronal activity. *European Journal of Neuroscience*, 16(5), 817–822. <https://doi.org/10.1046/j.1460-9568.2002.02150.x>
- Maier, S. F. (2015). Behavioral control blunts reactions to contemporaneous and future adverse events: Medial prefrontal cortex plasticity and a corticostriatal network. *Neurobiology of Stress*, 1, 12–22.
<https://doi.org/10.1016/j.ynstr.2014.09.003>
- Mansari, M. E., Sánchez, C., Chouvet, G., Renaud, B., & Haddjeri, N. (2005). Effects of Acute and Long-Term Administration of Escitalopram and Citalopram on Serotonin Neurotransmission: An In Vivo Electrophysiological Study in Rat Brain. *Neuropsychopharmacology*, 30(7), 1269–1277.
<https://doi.org/10.1038/sj.npp.1300686>
- Marazziti, D., Mucci, F., Tripodi, B., Carbone, M. G., Muscarella, A., Falaschi, V., & Baroni, S. (2019). Emotional Blunting, Cognitive Impairment, Bone Fractures, and Bleeding as Possible Side Effects of Long-Term Use of SSRIs. *Clinical Neuropsychiatry*, 16(2), 75–85.
- Marcinkiewicz, C. A., Mazzone, C. M., D'Agostino, G., Halladay, L. R., Hardaway, J. A., DiBerto, J. F., Navarro, M., Burnham, N. W., Cristiano, C., Dorrier, C. E.,

- Tipton, G. J., Ramakrishnan, C., Kozicz, T., Deisseroth, K., Thiele, T. E., McElligott, Z. A., Holmes, A., Heisler, L. K., & Kash, T. L. (2016). Serotonin engages an anxiety and fear-promoting circuit in the extended amygdala. *Nature*, 537(7618), 97–101. <https://doi.org/10.1038/nature19318>
- Marek, G. J., & Marek, G. J. (2010). Electrophysiology of Serotonin Receptors. *Handbook of Behavioral Neuroscience*, 21, 163–182. [https://doi.org/10.1016/s1569-7339\(10\)70077-1](https://doi.org/10.1016/s1569-7339(10)70077-1)
- Marinelli, S., Marinelli, S., Schnell, S. A., Hack, S. P., Christie, M. J., Wessendorf, M. W., & Vaughan, C. W. (2004). Serotonergic and Nonserotonergic Dorsal Raphe Neurons Are Pharmacologically and Electrophysiologically Heterogeneous. *Journal of Neurophysiology*, 92(6), 3532–3537. <https://doi.org/10.1152/jn.00437.2004>
- Masson, J., Emerit, M. B., Hamon, M., & Darmon, M. (2012). *Serotonergic signaling: Multiple effectors and pleiotropic effects*. 1(6), 685–713. <https://doi.org/10.1002/wmts.50>
- Maswood, S., Barter, J. E., Watkins, L. R., & Maier, S. F. (1998). Exposure to inescapable but not escapable shock increases extracellular levels of 5-HT in the dorsal raphe nucleus of the rat. *Brain Research*, 783(1), 115–120. [https://doi.org/10.1016/s0006-8993\(97\)01313-9](https://doi.org/10.1016/s0006-8993(97)01313-9)
- Matsumoto, M., Okihide Hikosaka, & Hikosaka, O. (2007). Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature*, 447(7148), 1111–1115. <https://doi.org/10.1038/nature05860>
- McCabe, C., Mishor, Z., Cowen, P. J., & Harmer, C. J. (2010). Diminished Neural Processing of Aversive and Rewarding Stimuli During Selective Serotonin

- Reuptake Inhibitor Treatment. *Biological Psychiatry*, 67(5), 439–445.
<https://doi.org/10.1016/j.biopsych.2009.11.001>
- McDevitt, R. A., Tiran-Cappello, A., Shen, H., Balderas, I., Britt, J. P., Marino, R. A. M., Rosa A.M. Marino, Chung, S. L., Richie, C. T., Harvey, B. K., & Bonci, A. (2014). Serotonergic versus Nonserotonergic Dorsal Raphe Projection Neurons: Differential Participation in Reward Circuitry. *Cell Reports*, 8(6), 1857–1869. <https://doi.org/10.1016/j.celrep.2014.08.037>
- Mengod, G., Cortés, R., Vilaró, M. T., & Hoyer, D. (2010). Distribution of 5-HT Receptors in the Central Nervous System. *Handbook of Behavioral Neuroscience*, 21, 123–138. [https://doi.org/10.1016/s1569-7339\(10\)70074-6](https://doi.org/10.1016/s1569-7339(10)70074-6)
- Michelsen, K. A., Prickaerts, J., & Steinbusch, H. W. M. (2008). The dorsal raphe nucleus and serotonin: Implications for neuroplasticity linked to major depression and Alzheimer's disease. *Progress in Brain Research*, 172, 233–264. [https://doi.org/10.1016/s0079-6123\(08\)00912-6](https://doi.org/10.1016/s0079-6123(08)00912-6)
- Michelsen, K. A., Schmitz, C., & Steinbusch, H. W. M. (2007). The dorsal raphe nucleus—From silver stainings to a role in depression. *Brain Research Reviews*, 55(2), 329–342. <https://doi.org/10.1016/j.brainresrev.2007.01.002>
- Miller, J. K., Ayzenshtat, I., Carrillo-Reid, L., & Yuste, R. (2014). Visual stimuli recruit intrinsically generated cortical ensembles. *Proceedings of the National Academy of Sciences*, 111(38), E4053–E4061. <https://doi.org/10.1073/pnas.1406077111>
- Miyazaki, K., Miyazaki, K. W., & Doya, K. (2012). The Role of Serotonin in the Regulation of Patience and Impulsivity. *Molecular Neurobiology*, 45(2), 213–224. <https://doi.org/10.1007/s12035-012-8232-6>

- Moncrieff, Ruth E. Cooper, Tom Stockmann, Simone Amendola, Michael P. Hengartner, & Mark A. Horowitz. (2022). The serotonin theory of depression: A systematic umbrella review of the evidence. *Molecular Psychiatry*. <https://doi.org/10.1038/s41380-022-01661-0>
- Montagne-Clavel, J., Oliveras, J. L., & Martin, G. (1995). Single-unit recordings at dorsal raphe nucleus in the awake-anesthetized rat: Spontaneous activity and responses to cutaneous innocuous and noxious stimulations. *Pain*, 60(3), 303–310. [https://doi.org/10.1016/0304-3959\(94\)00129-3](https://doi.org/10.1016/0304-3959(94)00129-3)
- Montalbano, A., Corradetti, R., & Mlinar, B. (2015). Pharmacological Characterization of 5-HT_{1A} Autoreceptor-Coupled GIRK Channels in Rat Dorsal Raphe 5-HT Neurons. *PLOS ONE*, 10(10). <https://doi.org/10.1371/journal.pone.0140369>
- Monti, J. M. (2010). The role of dorsal raphe nucleus serotonergic and non-serotonergic neurons, and of their receptors, in regulating waking and rapid eye movement (REM) sleep. *Sleep Medicine Reviews*, 14(5), 319–327. <https://doi.org/10.1016/j.smr.2009.10.003>
- Moret, C., & Briley, M. (2000). The possible role of 5-HT_{1B/D} receptors in psychiatric disorders and their potential as a target for therapy. *European Journal of Pharmacology*, 404(1), 1–12. [https://doi.org/10.1016/S0014-2999\(00\)00581-1](https://doi.org/10.1016/S0014-2999(00)00581-1)
- Nakahama, H., Shima, K., Yamamoto, M., & Aya, K. (1981). Regularity of the spontaneous discharge of neurons in the nucleus raphe dorsalis of the cat. *Neuroscience Letters*, 23(2), 161–165. [https://doi.org/10.1016/0304-3940\(81\)90034-3](https://doi.org/10.1016/0304-3940(81)90034-3)

- Nakamura, K. (2013). The role of the dorsal raphe nucleus in reward-seeking behavior. *Frontiers in Integrative Neuroscience*, 7, 60. <https://doi.org/10.3389/fnint.2013.00060>
- Newman, M. (2010). *Networks: An Introduction*. <https://doi.org/10.1093/acprof:oso/9780199206650.001.0001>
- Nicolelis, M. A. L., & Lebedev, M. A. (2009). Principles of neural ensemble physiology underlying the operation of brain–machine interfaces. *Nature Reviews Neuroscience*, 10(7), 530–540. <https://doi.org/10.1038/nrn2653>
- Niederkoefler, V., Asher, T. E., Okaty, B. W., Rood, B. D., Narayan, A., Hwa, L. S., Beck, S. G., Miczek, K. A., & Dymecki, S. M. (2016). Identification of Serotonergic Neuronal Modules that Affect Aggressive Behavior. *Cell Reports*, 17(8), 1934–1949. <https://doi.org/10.1016/j.celrep.2016.10.063>
- Nigam, S., Shimonono, M., Ito, S., Yeh, F.-C., Timme, N., Myroshnychenko, M., Lapis, C. C., Tosi, Z., Hottowy, P., Smith, W. C., Masmanidis, S. C., Litke, A. M., Sporns, O., & Beggs, J. M. (2016). Rich-Club Organization in Effective Connectivity among Cortical Neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 36(3), 670–684. <https://doi.org/10.1523/JNEUROSCI.2177-15.2016>
- Ochi, J., & Shimizu, K. (1978). Occurrence of dopamine-containing neurons in the midbrain raphe nuclei of the rat. *Neuroscience Letters*, 8(4), 317–320. [https://doi.org/10.1016/0304-3940\(78\)90142-8](https://doi.org/10.1016/0304-3940(78)90142-8)
- Oikonomou, G., Altermatt, M., Zhang, R., Zhang, R., Coughlin, G. M., Montz, C. J., Gradinaru, V., & Prober, D. A. (2019). The Serotonergic Raphe Promote Sleep in Zebrafish and Mice. *Neuron*, 103(4), 686. <https://doi.org/10.1016/j.neuron.2019.05.038>

- Okaty, B. W., Commons, K. G., & Dymecki, S. M. (2019). Embracing diversity in the 5-HT neuronal system. *Nature Reviews Neuroscience*, 20(7), 397–424. <https://doi.org/10.1038/s41583-019-0151-3>
- Okaty, B. W., Freret, M. E., Rood, B. D., Brust, R. D., Hennessy, M. L., deBairos, D., Kim, J. C., Cook, M. N., & Dymecki, S. M. (2015). Multi-Scale Molecular Deconstruction of the Serotonin Neuron System. *Neuron*, 88(4), 774–791. <https://doi.org/10.1016/j.neuron.2015.10.007>
- Okun, M. S., Steinmetz, N. A., Lak, A., Dervinis, M., Kenneth D Harris, & Harris, K. D. (2019). Distinct Structure of Cortical Population Activity on Fast and Infralow Timescales. *Cerebral Cortex*, 29(5), 2196–2210. <https://doi.org/10.1093/cercor/bhz023>
- Oliveras, J. L., Guilbaud, G., & Besson, J. M. (1979). A map of serotonergic structures involved in stimulation producing analgesia in unrestrained freely moving cats. *Brain Research*, 164(1), 317–322. [https://doi.org/10.1016/0006-8993\(79\)90029-5](https://doi.org/10.1016/0006-8993(79)90029-5)
- Ostojic, S., Brunel, N., & Hakim, V. (2009). How connectivity, background activity, and synaptic properties shape the cross-correlation between spike trains. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 29(33), 10234–10253. <https://doi.org/10.1523/JNEUROSCI.1275-09.2009>
- Pachitariu, M., Rossant, C., Steinmetz, N. A., Colonell, J., Adrian Gopnik Bondy, Olivier Winter, kushbanga, Bhagat, J., Mari Sosa, Dan O'Shea, Jose Guzman, Kouichi C. Nakamura, geffenlab, Paul Botros, Rajat Saxena, Alan Liddell, John Pellman, Martin Spacek, bryzgalovdm, ... Beau, M. (2021). *MouseLand/Kilosort: Kilosort 2.5*. <https://doi.org/10.5281/zenodo.4482749>

- Pachitariu, M., Steinmetz, N. A., Kadir, S., Carandini, M., H D Kenneth, & H, K. D. (2016). Kilosort: Realtime spike-sorting for extracellular electrophysiology with hundreds of channels. *BioRxiv*, 061481. <https://doi.org/10.1101/061481>
- Palacios, J., Palacios, J. M., & José M. Palacios. (2016). Serotonin receptors in brain revisited. *Brain Research*, 1645, 46–49. <https://doi.org/10.1016/j.brainres.2015.12.042>
- Paneri, S., & Gregoriou, G. G. (2017). Top-Down Control of Visual Attention by the Prefrontal Cortex. Functional Specialization and Long-Range Interactions. *Frontiers in Neuroscience*, 11, 545–545. <https://doi.org/10.3389/fnins.2017.00545>
- Paquelet, Kassandra Carrion, Clay O. Lacefield, Pengcheng Zhou, René Hen, & Bradley R. Miller. (2022). Single-cell activity and network properties of dorsal raphe nucleus serotonin neurons during emotionally salient behaviors. *Neuron*. <https://doi.org/10.1016/j.neuron.2022.05.015>
- Pedersen, E. J., Miller, D. L., Simpson, G., & Ross, N. (2018). *Hierarchical generalized additive models: An introduction with mgcv*. <https://doi.org/10.7287/peerj.preprints.27320v1>
- Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Bertrand Thirion, Thirion, B., Bertrand Thirion, Grisel, O., Blondel, M., Müller, A., Nothman, J., Joel Nothman, Louppe, G., Prettenhofer, P., Weiss, R., Dubourg, V., Vincent Dubourg, Vanderplas, J., Passos, A., ... Duchesnay, E. (2012). Scikit-learn: Machine Learning in Python. *ArXiv: Learning*, 1201. <https://doi.org/10.5555/1953048.2078195>
- Petrov, T., Krukoff, T. L., & Jhamandas, J. H. (1994). Chemically defined collateral projections from the pons to the central nucleus of the amygdala and

- hypothalamic paraventricular nucleus in the rat. *Cell and Tissue Research*, 277(2), 289–295. <https://doi.org/10.1007/BF00327776>
- Peyron, C., Petit, J. M., Rampon, C., Jouvet, M., & Luppi, P. H. (1998). Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. *Neuroscience*, 82(2), 443–468. [https://doi.org/10.1016/s0306-4522\(97\)00268-6](https://doi.org/10.1016/s0306-4522(97)00268-6)
- Pilling, S., Whittington, C., Taylor, C., & Kendrick, T. (2011). Identification and care pathways for common mental health disorders: Summary of NICE guidance. *BMJ*, 342(7808), 1203–1206. <https://doi.org/10.1136/bmj.d2868>
- Pillow, J. W., Shlens, J., Paninski, L., Sher, A., Litke, A. M., Chichilnisky, E. J., & Simoncelli, E. P. (2008). Spatio-temporal correlations and visual signalling in a complete neuronal population. *Nature*, 454(7207), 995–999. <https://doi.org/10.1038/nature07140>
- Pinault, D. (1996). A novel single-cell staining procedure performed in vivo under electrophysiological control: Morpho-functional features of juxtacellularly labeled thalamic cells and other central neurons with biocytin or Neurobiotin. *Journal of Neuroscience Methods*, 65(2), 113–136. [https://doi.org/10.1016/0165-0270\(95\)00144-1](https://doi.org/10.1016/0165-0270(95)00144-1)
- Prouty, E. W., Chandler, D. J., & Waterhouse, B. D. (2017). Neurochemical differences between target-specific populations of rat dorsal raphe projection neurons. *Brain Research*, 1675, 28–40. <https://doi.org/10.1016/j.brainres.2017.08.031>
- Puig, M. V., & Gullledge, A. T. (2011). Serotonin and prefrontal cortex function: Neurons, networks, and circuits. *Molecular Neurobiology*, 44(3), 449–464. <https://doi.org/10.1007/s12035-011-8214-0>

- Qi, J., Zhang, S., Wang, H.-L., Wang, H., Jose de Jesus Aceves Buendia, de Jesus Aceves Buendia, J., Hoffman, A. F., Lupica, C. R., Seal, R. P., & Morales, M. (2014). A glutamatergic reward input from the dorsal raphe to ventral tegmental area dopamine neurons. *Nature Communications*, 5(1), 5390–5390. <https://doi.org/10.1038/ncomms6390>
- Ranade, S. P., & Mainen, Z. F. (2009). Transient firing of dorsal raphe neurons encodes diverse and specific sensory, motor, and reward events. *Journal of Neurophysiology*, 102(5), 3026–3037. <https://doi.org/10.1152/jn.00507.2009>
- Rapport, M. M., Arda Alden Green, Green, A. A., & Page, I. H. (1948). Partial purification of the vasoconstrictor in beef serum. *Journal of Biological Chemistry*, 174(2), 735–741. [https://doi.org/10.1016/s0021-9258\(18\)57355-5](https://doi.org/10.1016/s0021-9258(18)57355-5)
- Raymond, J. R. (1991). Protein kinase C induces phosphorylation and desensitization of the human 5-HT_{1A} receptor. *The Journal of Biological Chemistry*, 266(22), 14747–14753.
- Raymond, J. R., Mukhin, Y. V., Gettys, T. W., & Garnovskaya, M. N. (1999). The recombinant 5-HT_{1A} receptor: G protein coupling and signalling pathways. *British Journal of Pharmacology*, 127(8), 1751–1764. <https://doi.org/10.1038/sj.bjp.0702723>
- Ren, J., Friedmann, D., Xiong, J., Liu, C. D., Ferguson, B. R., Weerakkody, T., DeLoach, K. E., Ran, C., Pun, A., Sun, Y., Yanwen Sun, Weissbourd, B., Neve, R. L., Huguenard, J. R., Horowitz, M., & Luo, L. (2018). Anatomically Defined and Functionally Distinct Dorsal Raphe Serotonin Sub-systems. *Cell*, 175(2), 472–487. <https://doi.org/10.1016/j.cell.2018.07.043>

- Ren, J., Isakova, A., Friedmann, D., Zeng, J., Jiawei Zeng, Grutzner, S. M., Pun, A., Zhao, G. Q., Kolluru, S. S., Wang, R., Lin, R., Pengcheng Li, Pengcheng Li, Li, P., Anan Li, Li, A., Raymond, J. L., Luo, Q., Luo, M., ... Luo, L. (2019). Single-cell transcriptomes and whole-brain projections of serotonin neurons in the mouse dorsal and median raphe nuclei. *ELife*, 8. <https://doi.org/10.7554/elife.49424>
- Ressler, K. J., & Nemeroff, C. B. (2000). Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Depression and Anxiety*, 12, 2–19. [https://doi.org/10.1002/1520-6394\(2000\)12:1+<2::aid-da2>3.0.co;2-4](https://doi.org/10.1002/1520-6394(2000)12:1+<2::aid-da2>3.0.co;2-4)
- Riad, M., Garcia, S., Watkins, K. C., Jodoin, N., Doucet, E., Langlois, X., Mestikawy, S. E., Hamon, M., & Descarries, L. (2000). Somatodendritic localization of 5-HT_{1A} and preterminal axonal localization of 5-HT_{1B} serotonin receptors in adult rat brain. *The Journal of Comparative Neurology*, 417(2), 181–194. [https://doi.org/10.1002/\(sici\)1096-9861\(20000207\)417:2<181::aid-cne4>3.0.co;2-a](https://doi.org/10.1002/(sici)1096-9861(20000207)417:2<181::aid-cne4>3.0.co;2-a)
- Richardson-Jones, J. W., Craige, C. P., Nguyen, T. H., Kung, H. F., Gardier, A. M., Dranovsky, A., David, D. J., Guiard, B. P., Beck, S. G., Hen, R., & Leonardo, E. D. (2011). Serotonin-1A autoreceptors are necessary and sufficient for the normal formation of circuits underlying innate anxiety. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 31(16), 6008–6018. <https://doi.org/10.1523/JNEUROSCI.5836-10.2011>
- Rigotti, M., Barak, O., Warden, M. R., Wang, X. J., Daw, N. D., Miller, E. K., & Fusi, S. (2013). The importance of mixed selectivity in complex cognitive tasks. *Nature*, 497(7451), 585–590. <https://doi.org/10.1038/nature12160>

- Ritchie, J. B., Kaplan, D. M., & Klein, C. (2017). Decoding the Brain: Neural Representation and the Limits of Multivariate Pattern Analysis in Cognitive Neuroscience. *The British Journal for the Philosophy of Science*, 70(2), 581–607. <https://doi.org/10.1093/bjps/axx023>
- Roche, M., Commons, K. G., Peoples, A., & Valentino, R. J. (2003). Circuitry underlying regulation of the serotonergic system by swim stress. *The Journal of Neuroscience*, 23(3), 970–977. <https://doi.org/10.1523/jneurosci.23-03-00970.2003>
- Roiser, J. P., Levy, J., Fromm, S. J., Wang, H., Hasler, G., Sahakian, B. J., & Drevets, W. C. (2008). The effect of acute tryptophan depletion on the neural correlates of emotional processing in healthy volunteers. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, 33(8), 1992–2006. <https://doi.org/10.1038/sj.npp.1301581>
- Rozeske, R. R., Evans, A. K., Liu, G., Frank, M. G., Watkins, L. R., Lowry, C. A., & Maier, S. F. (2011). Uncontrollable, But Not Controllable, Stress Desensitizes 5-HT_{1A} Receptors in the Dorsal Raphe Nucleus. *The Journal of Neuroscience*, 31(40), 14107–14115. <https://doi.org/10.1523/jneurosci.3095-11.2011>
- Runyan, C. A., Piasini, E., Panzeri, S., & Harvey, C. D. (2017). Distinct timescales of population coding across cortex. *Nature*, 548(7665), 92–96. <https://doi.org/10.1038/nature23020>
- Rutter, J. J., Gundlach, C., & Auerbach, S. B. (1994). Increase in extracellular serotonin produced by uptake inhibitors is enhanced after chronic treatment with fluoxetine. *Neuroscience Letters*, 171(1), 183–186. [https://doi.org/10.1016/0304-3940\(94\)90635-1](https://doi.org/10.1016/0304-3940(94)90635-1)

- Sakai, K. (2011). Sleep-waking discharge profiles of dorsal raphe nucleus neurons in mice. *Neuroscience*, 197, 200–224.
<https://doi.org/10.1016/j.neuroscience.2011.09.024>
- Sakai, K., & Crochet, S. (2001). Differentiation of presumed serotonergic dorsal raphe neurons in relation to behavior and wake-sleep states. *Neuroscience*, 104(4), 1141–1155. [https://doi.org/10.1016/s0306-4522\(01\)00103-8](https://doi.org/10.1016/s0306-4522(01)00103-8)
- Sakai, K., Salvert, D., Touret, M., Touret, M., & Jouvet, M. (1977). Afferent connections of the nucleus raphe dorsalis in the cat as visualized by the horseradish peroxidase technique. *Brain Research*, 137(1), 11–35.
[https://doi.org/10.1016/0006-8993\(77\)91010-1](https://doi.org/10.1016/0006-8993(77)91010-1)
- Sales, A. C., G.W.T. Newton, Pickering, A. E., Dunham, J. P., & Jim Dunham. (2021). Multisite silicon probes enable simultaneous recording of spontaneous and evoked activity in multiple isolated C-fibres in rat saphenous nerve. *Journal of Neuroscience Methods*, 109419.
<https://doi.org/10.1016/j.jneumeth.2021.109419>
- Saxena, S., & Cunningham, J. P. (2019). Towards the neural population doctrine. *Current Opinion in Neurobiology*, 55, 103–111.
<https://doi.org/10.1016/j.conb.2019.02.002>
- Saxena, S., Russo, A. A., Cunningham, J. P., Cunningham, J. P., Churchland, M. M., Mark M. Churchland, & Mark M. Churchland. (2021). Motor cortex activity across movement speeds is predicted by network-level strategies for generating muscle activity. *BioRxiv*.
<https://doi.org/10.1101/2021.02.01.429168>
- Schneeberger, M., Parolari, L., Banerjee, T. D., Bhawe, V., Wang, P., Patel, B., Topilko, T., Wu, Z., Choi, C. H. J., Yu, X., Pellegrino, K., Engel, E. A., Cohen, P., Renier,

- N., Friedman, J. M., & Nectow, A. R. (2019). Regulation of Energy Expenditure by Brainstem GABA Neurons. *Cell*, 178(3), 672-685.e12. <https://doi.org/10.1016/j.cell.2019.05.048>
- Schweimer, J. V., Mallet, N., Sharp, T., & Ungless, M. A. (2011). Spike-timing relationship of neurochemically-identified dorsal raphe neurons during cortical slow oscillations. *Neuroscience*, 196, 115–123. <https://doi.org/10.1016/j.neuroscience.2011.08.072>
- Schweimer, J. V., & Ungless, M. A. (2010). Phasic responses in dorsal raphe serotonin neurons to noxious stimuli. *Neuroscience*, 171(4), 1209–1215. <https://doi.org/10.1016/j.neuroscience.2010.09.058>
- Scuvée-Moreau, J. J., & Dresse, A. E. (1979). Effect of various antidepressant drugs on the spontaneous firing rate of locus coeruleus and dorsal raphe neurons of the rat. *European Journal of Pharmacology*, 57(2–3), 219–225. [https://doi.org/10.1016/0014-2999\(79\)90368-6](https://doi.org/10.1016/0014-2999(79)90368-6)
- Seabold, S., Josef Perktold, & Perktold, J. (2010). *Statsmodels: Econometric and Statistical Modeling with Python*. 92–96. <https://doi.org/10.25080/majora-92bf1922-011>
- Sego, C., de Jesus Gonçalves, L., Lima, L., Furigo, I. C., Donato, J., & Metzger, M. (2014). Lateral habenula and the rostromedial tegmental nucleus innervate neurochemically distinct subdivisions of the dorsal raphe nucleus in the rat. *The Journal of Comparative Neurology*, 522(7), 1454–1484. <https://doi.org/10.1002/cne.23533>
- Sengupta, A., Bocchio, M., Bannerman, D. M., Sharp, T., & Capogna, M. (2017). Control of Amygdala Circuits by 5-HT Neurons via 5-HT and Glutamate

- Cotransmission. *The Journal of Neuroscience*, 37(7), 1785–1796.
<https://doi.org/10.1523/jneurosci.2238-16.2016>
- Sengupta, A., & Holmes, A. (2019). A Discrete Dorsal Raphe to Basal Amygdala 5-HT Circuit Calibrates Aversive Memory. *Neuron*, 103(3), 489.
<https://doi.org/10.1016/j.neuron.2019.05.029>
- Shahryar Noei, Ioannis S. Zouridis, Nikos K. Logothetis, Panzeri, S., & Totah, N. K. (2022). Distinct ensembles in the noradrenergic locus coeruleus are associated with diverse cortical states. *Proceedings of the National Academy of Sciences of the United States of America*, 119(18).
<https://doi.org/10.1073/pnas.2116507119>
- Sharp, T., & Barnes, N. M. (2020). Central 5-HT receptors and their function; present and future. *Neuropharmacology*, 177, 108155.
<https://doi.org/10.1016/j.neuropharm.2020.108155>
- Sharp, T., Boothman, L. J., Raley, J., & Quérée, P. (2007). Important messages in the ‘post’: Recent discoveries in 5-HT neurone feedback control. *Trends in Pharmacological Sciences*, 28(12), 629–636.
<https://doi.org/10.1016/j.tips.2007.10.009>
- Shelton, R. C., & Tomarken, A. J. (2001). Can recovery from depression be achieved? *Psychiatric Services (Washington, D.C.)*, 52(11), 1469–1478.
<https://doi.org/10.1176/appi.ps.52.11.1469>
- Shenoy, K. V., Meeker, D., Cao, S., Kureshi, S. A., Pesaran, B., Buneo, C. A., Batista, A. P., Mitra, P. P., Burdick, J. W., & Andersen, R. A. (2003). Neural prosthetic control signals from plan activity. *Neuroreport*, 14(4), 591–596.
<https://doi.org/10.1097/00001756-200303240-00013>

- Siegle, J. H., Jia, X., Durand, S., Gale, S., Bennett, C., Corbett Bennett, Graddis, N., Heller, G., Ramirez, T. K., Hannah Choi, Choi, H., Luviano, J., Groblewski, P. A., Ahmed, R., Arkhipov, A., Bernard, A., Billeh, Y. N., Brown, D., D, B., ... Koch, C. (2021). Survey of spiking in the mouse visual system reveals functional hierarchy. *Nature*, *592*(7852), 86–92. <https://doi.org/10.1038/s41586-020-03171-x>
- Siegle, J. H., López, A. C., Patel, Y. A., Abramov, K., Ohayon, S., & Voigts, J. (2017). Open Ephys: An open-source, plugin-based platform for multichannel electrophysiology. *Journal of Neural Engineering*, *14*(4), 045003–045003. <https://doi.org/10.1088/1741-2552/aa5eea>
- Simon, S. A., de Araujo, I. E., Gutierrez, R., & Nicolelis, M. A. L. (2006). The neural mechanisms of gustation: A distributed processing code. *Nature Reviews Neuroscience*, *7*(11), 890–901. <https://doi.org/10.1038/nrn2006>
- Singh, A., & Lesica, N. A. (2010). Incremental mutual information: A new method for characterizing the strength and dynamics of connections in neuronal circuits. *PLoS Computational Biology*, *6*(12), e1001035. <https://doi.org/10.1371/journal.pcbi.1001035>
- Smith, M. A., Jia, X., Zandvakili, A., & Kohn, A. (2013). Laminar dependence of neuronal correlations in visual cortex. *Journal of Neurophysiology*, *109*(4), 940–947. <https://doi.org/10.1152/jn.00846.2012>
- Smith, M. A., & Kohn, A. (2008). Spatial and Temporal Scales of Neuronal Correlation in Primary Visual Cortex. *The Journal of Neuroscience*, *28*(48), 12591–12603. <https://doi.org/10.1523/JNEUROSCI.2929-08.2008>
- Sotelo, C., Cholley, B., El Mestikawy, S., Gozlan, H., & Hamon, M. (1990). Direct Immunohistochemical Evidence of the Existence of 5-HT_{1A} Autoreceptors on

- Serotonergic Neurons in the Midbrain Raphe Nuclei. *European Journal of Neuroscience*, 2(12), 1144–1154. <https://doi.org/10.1111/j.1460-9568.1990.tb00026.x>
- Spaethling, J. M., Piel, D. A., Piel, D. A., Dueck, H., Buckley, P. T., Buckley, P. T., Morris, J., Fisher, S. A., Lee, J., Sul, J.-Y., Kim, J., Bartfai, T., Beck, S. G., & Eberwine, J. (2014). Serotonergic neuron regulation informed by in vivo single-cell transcriptomics. *The FASEB Journal*, 28(2), 771–780. <https://doi.org/10.1096/fj.13-240267>
- Sprouse, J. S., & Aghajanian, G. K. (1987). Electrophysiological responses of serotonergic dorsal raphe neurons to 5-HT_{1A} and 5-HT_{1B} agonists. *Synapse (New York, N.Y.)*, 1(1), 3–9. <https://doi.org/10.1002/syn.890010103>
- Steinbusch, H. W. (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience*, 6(4), 557–618. [https://doi.org/10.1016/0306-4522\(81\)90146-9](https://doi.org/10.1016/0306-4522(81)90146-9)
- Steinmetz, N. A., Koch, C., Harris, K. D. M., Kenneth D Harris, Harris, K. D., & Carandini, M. (2018). Challenges and opportunities for large-scale electrophysiology with Neuropixels probes. *Current Opinion in Neurobiology*, 50, 92–100. <https://doi.org/10.1016/j.conb.2018.01.009>
- Steinmetz, N. A., Zatka-Haas, P., Carandini, M., Harris, K. D., & Harris, K. D. M. (2019). Distributed coding of choice, action and engagement across the mouse brain. *Nature*, 576(7786), 266–273. <https://doi.org/10.1038/s41586-019-1787-x>
- Stevenson, I. H., & Kording, K. P. (2011). How advances in neural recording affect data analysis. *Nature Neuroscience*, 14(2), 139–142. <https://doi.org/10.1038/nn.2731>

- Stringer, C., Michaelos, M., Tsyboulski, D., Lindo, S. E., & Pachitariu, M. (2021). High-precision coding in visual cortex. *Cell*, 184(10), 2767-2778.e15. <https://doi.org/10.1016/j.cell.2021.03.042>
- Svensson, T. H., Bunney, B. S., & Aghajanian, G. K. (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Research*, 92(2), 291–306. [https://doi.org/10.1016/0006-8993\(75\)90276-0](https://doi.org/10.1016/0006-8993(75)90276-0)
- Takase, L. F., Nogueira, M. I., Baratta, M., Baratta, M. V., Bland, S. T., Watkins, L. R., Maier, S. F., Fornal, C. A., & Jacobs, B. L. (2004). Inescapable shock activates serotonergic neurons in all raphe nuclei of rat. *Behavioural Brain Research*, 153(1), 233–239. <https://doi.org/10.1016/j.bbr.2003.12.020>
- Tamir, H., & Gershon, M. D. (1990). Serotonin-Storing Secretory Vesicles. *Annals of the New York Academy of Sciences*, 600(1), 53–67. <https://doi.org/10.1111/j.1749-6632.1990.tb16872.x>
- Thivierge, J.-P. (2014). Scale-free and economical features of functional connectivity in neuronal networks. *Physical Review. E, Statistical, Nonlinear, and Soft Matter Physics*, 90(2), 022721. <https://doi.org/10.1103/PhysRevE.90.022721>
- Tignol, J. (1993). A double-blind, randomized, fluoxetine-controlled multicenter study of paroxetine in the treatment of depression. *Journal of Clinical Psychopharmacology*, 13(6). <https://doi.org/10.1097/00004714-199312002-00003>
- Tohen, M., Vieta, E., Calabrese, J., Ketter, T. A., Sachs, G., Bowden, C., Mitchell, P. B., Centorrino, F., Risser, R., Baker, R. W., Evans, A. R., Beymer, K., Dube, S., Tollefson, G. D., & Breier, A. (2003). Efficacy of olanzapine and olanzapine-fluoxetine combination in the treatment of bipolar I depression. *Archives of*

- General Psychiatry*, 60(11), 1079–1088.
<https://doi.org/10.1001/archpsyc.60.11.1079>
- Totah, N. K. (2018). The locus coeruleus: A complex and differentiated neuromodulatory system. *Neuron*.
<https://doi.org/10.1016/j.neuron.2018.07.037>
- Totah, N. K., Logothetis, N. K., & Eschenko, O. (2019). Noradrenergic ensemble-based modulation of cognition over multiple timescales. *Brain Research*, 1709, 50–66. <https://doi.org/10.1016/j.brainres.2018.12.031>
- Totah, N. K., Neves, R. M., Panzeri, S., Logothetis, N. K., & Eschenko, O. (2018). The Locus Coeruleus Is a Complex and Differentiated Neuromodulatory System. *Neuron*, 99(5), 1055-1068.e6. <https://doi.org/10.1016/j.neuron.2018.07.037>
- Trulson, M. E., & Jacobs, B. L. (1979). Raphe unit activity in freely moving cats: Correlation with level of behavioral arousal. *Brain Research*, 163(1), 135–150.
[https://doi.org/10.1016/0006-8993\(79\)90157-4](https://doi.org/10.1016/0006-8993(79)90157-4)
- Twarog, B. M. (1954). Responses of a molluscan smooth muscle to acetylcholine and 5-hydroxytryptamine. *Journal of Cellular and Comparative Physiology*, 44(1), 141–163. <https://doi.org/10.1002/jcp.1030440112>
- Twarog, B. M., & Page, I. H. (1953). Serotonin Content of Some Mammalian Tissues and Urine and a Method for Its Determination. *American Journal of Physiology*, 175(1), 157–161. <https://doi.org/10.1152/ajplegacy.1953.175.1.157>
- Ulyanova, A. V., Cottone, C., Adam, C. D., Gagnon, K. G., Cullen, D. K., Holtzman, T., Jamieson, B. G., Koch, P. F., Chen, H. I., Johnson, V. E., & Wolf, J. A. (2019). Multichannel Silicon Probes for Awake Hippocampal Recordings in Large Animals. *Frontiers in Neuroscience*, 13, 397.
<https://doi.org/10.3389/fnins.2019.00397>

- Urai, A., Doiron, B., Leifer, A., & Churchland, A. (2021). Large-scale neural recordings call for new insights to link brain and behavior. *Nature Neuroscience*. <https://doi.org/10.1038/s41593-021-00980-9>
- Urbain, N., Nadia Urbain, Urbain, N., Creamer, K., & Debonnel, G. (2006). Electrophysiological diversity of the dorsal raphe cells across the sleep–wake cycle of the rat. *The Journal of Physiology*, 573(3), 679–695. <https://doi.org/10.1113/jphysiol.2006.108514>
- Urch, C. E., Donovan-Rodriguez, T., & Dickenson, A. H. (2003). Alterations in dorsal horn neurones in a rat model of cancer-induced bone pain. *Pain*, 106(3), 347–356. <https://doi.org/10.1016/j.pain.2003.08.002>
- Vallat, R. & Raphael Vallat. (2018). Pingouin: Statistics in Python. *Journal of Open Source Software*, 3(31), 1026. <https://doi.org/10.21105/joss.01026>
- Vandermaelen, C. P., & Aghajanian, G. K. (1983). Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. *Brain Research*, 289(1), 109–119. [https://doi.org/10.1016/0006-8993\(83\)90011-2](https://doi.org/10.1016/0006-8993(83)90011-2)
- Varga, V., Kocsis, B., & Sharp, T. (2003). Electrophysiological evidence for convergence of inputs from the medial prefrontal cortex and lateral habenula on single neurons in the dorsal raphe nucleus. *The European Journal of Neuroscience*, 17(2), 280–286. <https://doi.org/10.1046/j.1460-9568.2003.02465.x>
- Varga, V., Székely, A. D., Csillag, A., Sharp, T., & Hajós, M. (2001). Evidence for a role of GABA interneurons in the cortical modulation of midbrain 5-hydroxytryptamine neurones. *Neuroscience*, 106(4), 783–792. [https://doi.org/10.1016/s0306-4522\(01\)00294-9](https://doi.org/10.1016/s0306-4522(01)00294-9)

- Vergé, D., Daval, G., Patey, A., Gozlan, H., Mestikawy, S. E., Mestikawy, S. E., Mestikawy, S. E., Hamon, M., & Hamon, M. (1985). Presynaptic 5-HT autoreceptors on serotonergic cell bodies and/or dendrites but not terminals are of the 5-HT_{1A} subtype. *European Journal of Pharmacology*, 113(3), 463–464. [https://doi.org/10.1016/0014-2999\(85\)90099-8](https://doi.org/10.1016/0014-2999(85)90099-8)
- Virtanen, P., Gommers, R., Burovski, E., Oliphant, T. E., Weckesser, W., Cournapeau, D., alexbr, Peterson, P., Wilson, J., Reddy, T., Mayorov, N., Haberland, M., Nelson, A. T., Nelson, A., van der Walt, S., Laxalde, D., Brett, M., Larson, E. B., Polat, I., ... Feng, Y. (2020). *scipy/scipy: SciPy 1.5.2*. <https://doi.org/10.5281/zenodo.3958354>
- Voigts, J., Pritchett, D. L., Moore, C. I., Siegle, J. H., & Joshua H. Siegle. (2013). The flexDrive: An ultra-light implant for optical control and highly parallel chronic recording of neuronal ensembles in freely moving mice. *Frontiers in Systems Neuroscience*. <https://doi.org/10.3389/fnsys.2013.00008>
- Walther, D. J., Peter, J.-U., Bashammakh, S., Hörtnagl, H., Voits, M., Fink, H., & Bader, M. (2003). Synthesis of Serotonin by a Second Tryptophan Hydroxylase Isoform. *Science*, 299(5603), 76–76. <https://doi.org/10.1126/science.1078197>
- Wang, N., Zhang, Y., Wang, J.-Y., Gao, G., & Luo, F. (2010). Effects of pentobarbital anesthesia on nociceptive processing in the medial and lateral pain pathways in rats. *Neuroscience Bulletin*, 26(3), 188–196. <https://doi.org/10.1007/s12264-010-0150-x>
- Wang, Q. P., & Nakai, Y. (1994). The dorsal raphe: An important nucleus in pain modulation. *Brain Research Bulletin*, 34(6), 575–585. [https://doi.org/10.1016/0361-9230\(94\)90143-0](https://doi.org/10.1016/0361-9230(94)90143-0)

- Wang, Q. P., Ochiai, H., & Nakai, Y. (1992). GABAergic innervation of serotonergic neurons in the dorsal raphe nucleus of the rat studied by electron microscopy double immunostaining. *Brain Research Bulletin*, 29(6), 943–948. [https://doi.org/10.1016/0361-9230\(92\)90169-x](https://doi.org/10.1016/0361-9230(92)90169-x)
- Warden, D., Rush, A. J., Trivedi, M. H., Fava, M., & Wisniewski, S. R. (2007). The STAR*D project results: A comprehensive review of findings. *Current Psychiatry Reports*, 9(6), 449–459. <https://doi.org/10.1007/s11920-007-0061-3>
- Warden, M. R., Selimbeyoglu, A., Mirzabekov, J. J., Lo, M., Thompson, K. R., Kim, S.-Y., Adhikari, A., Tye, K. M., Frank, L. M., & Deisseroth, K. (2012). A prefrontal cortex-brainstem neuronal projection that controls response to behavioural challenge. *Nature*, 492(7429), 428–432. <https://doi.org/10.1038/nature11617>
- Warren, J. (2020). The trouble with antidepressants: Why the evidence overplays benefits and underplays risks-an essay by John B Warren. *BMJ*, 370. <https://doi.org/10.1136/bmj.m3200>
- Waselus, M., Valentino, R. J., & Van Bockstaele, E. J. (2011). Collateralized dorsal raphe nucleus projections: A mechanism for the integration of diverse functions during stress. *Journal of Chemical Neuroanatomy*, 41(4), 266–280. <https://doi.org/10.1016/j.jchemneu.2011.05.011>
- Waterhouse, B. D., Devilbiss, D. M., Seiple, S., & Markowitz, R. S. (2004). Sensorimotor-related discharge of simultaneously recorded, single neurons in the dorsal raphe nucleus of the awake, unrestrained rat. *Brain Research*, 1000(1), 183–191. <https://doi.org/10.1016/j.brainres.2003.11.030>

- Weissbourd, B., Ren, J., DeLoach, K. E., Guenthner, C. J., Miyamichi, K., & Luo, L. (2014). Presynaptic partners of dorsal raphe serotonergic and GABAergic neurons. *Neuron*, 83(3), 645–662. <https://doi.org/10.1016/j.neuron.2014.06.024>
- Westlund, K. N., Denney, R. M., Rose, R. M., C.W. Abell, & Abell, C. W. (1988). Localization of distinct monoamine oxidase A and monoamine oxidase B cell populations in human brainstem. *Neuroscience*, 25(2), 439–456. [https://doi.org/10.1016/0306-4522\(88\)90250-3](https://doi.org/10.1016/0306-4522(88)90250-3)
- Whitaker-Azmitia, P. M. (1999). The Discovery of Serotonin and its Role in Neuroscience. *Neuropsychopharmacology*, 21(2), 2–8. [https://doi.org/10.1016/s0893-133x\(99\)00031-7](https://doi.org/10.1016/s0893-133x(99)00031-7)
- Wiklund, L., Léger, L., & Persson, M. (1981). Monoamine cell distribution in the cat brain stem. A fluorescence histochemical study with quantification of indolaminergic and locus coeruleus cell groups. *The Journal of Comparative Neurology*, 203(4), 613–647. <https://doi.org/10.1002/cne.902030405>
- Wilkinson, L. O., & Jacobs, B. L. (1988). Lack of response of serotonergic neurons in the dorsal raphe nucleus of freely moving cats to stressful stimuli. *Experimental Neurology*, 101(3), 445–457. [https://doi.org/10.1016/0014-4886\(88\)90055-6](https://doi.org/10.1016/0014-4886(88)90055-6)
- Woolley, D. W., & Shaw, E. (1954). A BIOCHEMICAL AND PHARMACOLOGICAL SUGGESTION ABOUT CERTAIN MENTAL DISORDERS. *Proceedings of the National Academy of Sciences of the United States of America*, 40(4), 228–231.
- Xu, Z., Feng, Z., Zhao, M., Sun, Q., Deng, L., Jia, X., Jiang, T., Luo, P., Chen, W., Tudi, A., Yuan, J., Li, X., Gong, H., Luo, Q., & Li, A. (2021). Whole-brain connectivity

atlas of glutamatergic and GABAergic neurons in the mouse dorsal and median raphe nuclei. *ELife*, 10, e65502. <https://doi.org/10.7554/eLife.65502>

Yatziv, S.-L., Yudco, O., Dickmann, S., & Devor, M. (2020). Patterns of neural activity in the mouse brain: Wakefulness vs. General anesthesia. *Neuroscience Letters*, 735, 135212. <https://doi.org/10.1016/j.neulet.2020.135212>

Yoshioka, M., Matsumoto, M., Togashi, H., & Saito, H. (1995). Effects of conditioned fear stress on 5-HT release in the rat prefrontal cortex. *Pharmacology, Biochemistry and Behavior*, 51(2), 515–519. [https://doi.org/10.1016/0091-3057\(95\)00045-x](https://doi.org/10.1016/0091-3057(95)00045-x)

Yuste, R. (2015). From the neuron doctrine to neural networks. *Nature Reviews. Neuroscience*, 16(8), 487–497. <https://doi.org/10.1038/nrn3962>