Investigating the Role of the Lateral Nigrostriatal Pathway in Modulating Voluntary Movement

By

Penelope Anne Young

A Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

in the Division of Medical Sciences (Neuroscience)

© Penelope Anne Young, 2023
University of Victoria

All rights reserved. This thesis may not be reproduced in whole or in part, by photography or other means, without the permission of the author.

Investigating the Role of the Lateral Nigrostriatal Pathway in
Modulating Voluntary Movement

By

Penelope Anne Young

Supervisory Committee:

Dr. Raad Nashmi, Supervisor
Department of Biology

Dr. Kerry R. Delaney, Outside Member
Department of Biology

Dr. Craig E. Brown, Departmental Member
Division of Medical Sciences

Abstract

The scientific community has known for decades that nigrostriatal dopamine is required for the initiation of voluntary movement. The dogma has stated that tonic release of dopamine in the striatum, the input center of the basal ganglia, acts as a permissive signal for voluntary movement. However, there is increasing evidence that transient dopaminergic signals are not only involved in reinforcing learning and reward, but also in actively modulating voluntary movement. How exactly transient, or phasic, dopaminergic signals modulate voluntary action is still unclear, in large part due to the heterogeneity of midbrain dopaminergic neurons (DANs) and downstream striatal subregions, and the number of functions phasic dopamine signalling is involved in, including reward prediction error (RPE) encoding, encoding of novel stimuli, and attributing value to stimuli.

There is evidence that suggests that the nigrostriatal pathway may be functionally separated along medial and lateral pathways, and consequently that the lateral pathway, namely the lateral substantia nigra pars compacta (SNCL) and the lateral dorsal striatum (DLS), may have a shared functionality in modulating voluntary movement. Although a number of studies have optogenetically stimulated either SNC DAN somata or DAN axon terminals in the DLS, no study to our knowledge has attempted to link the SNCL and DLS functionally regarding voluntary movement. Furthermore, to our knowledge no study has attempted to investigate the role of phasic lateral nigrostriatal dopaminergic signalling in behaviours beyond sub-sec/secs of locomotion, or how the potentially movement-promoting signal of phasic midbrain dopaminergic activity intersects with the novelty encoding function of SNC dopamine transients.

To address these gaps in our understanding on how lateral nigrostriatal phasic dopamine modulates voluntary movement, we generated a transgenic mouse line conditionally expressing a light-sensitive channel protein (channelrhodopsin-2, ChR2) in DANs, which would depolarize the neuron when exposed to blue light. We implanted optic fibres over the SNCL or over the DLS and stimulated mice while they explored an open field (OF). The mice were stimulated non-continuously at 12.5Hz, which mimics endogenous DAN phasic activity, and their activity measured for a total of 40 min. The mice were then re-introduced to the same OF and given the same stimulation protocol the next day. We found that phasic stimulation of SNCL somata increased exploratory activity, including rate of horizontal movement, locomotion, and rearing, but not grooming, during the stimulation periods of the first OF day, but not on the second OF day.

Specifically, stimulation on the first day increased the number of locomotion initiations without increasing the vigour of initiation. In contrast, on the second OF day we did not observe a stimulus-correlated increase in exploratory activity, but did observe a stimulus-dependant decrease in habituation relative to control (non ChR2-expressing) animals. Stimulation of DAN axons on either OF day in the DLS had no effect on activity. Our findings suggest that phasic activity of SNCL DANs promotes exploratory behaviours in a novelty-dependant manner and may prevent habituation to stimuli of lower novelty.

Table of Contents

Supervisory Committee: ii
Abstract iii
Table of Contents v
List of Figures viii
List of Tables x
List of Abbreviations xi
Acknowledgements xii
Chapter 1. Introduction 1
1.1 Background and Rationale 1
1.2 The Basal Ganglia: An Overview 1
1.2.1 The Direct and Indirect pathway 2
1.2.1.1 Dopamine Receptors 4
1.2.2 The Dorsal Striatum 5
1.2.3 The Dopaminergic Midbrain 6
1.3 The Substantia Nigra Pars Compacta 7
1.3.1 Dopamine Neurons of the Substantia Nigra Pars Compacta: An Overview 7
1.3.2 SNC Dopamine Neuron Firing Characteristics 8
1.3.3 Dopaminergic Neuronal Heterogeneity Across Different Spatial Regions of the SNC 8
1.4 Substantia Nigral Dopamine and Movement 9
1.4.1 The Reward Prediction Error Hypothesis 10
1.4.2 Phasic Dopamine Encodes Elements of Movement 11
1.4.2.1 The Role of Dopamine in Movement Onset 11
1.4.2.2 The Role of Dopamine in Movement Vigour 12
1.4.2.3 Role of Dopamine in Movement Refinement 13
1.5 Functional Segregation of the Lateral Nigrostriatal Pathway 14
1.6 Dopamine Signalling and the Novel Environment 14
1.6.1 Novelty Encoding 14
1.6.2 Novelty Seeking 15
1.7 The Open Field 16
1.7.1 An Overview 16
1.7.2 Habituation 17

1.7.3. Dopamine and Habituation 17
1.8 Research Objectives and Hypothesis 18
Chapter 2. Materials and Methods 19
2.1 Experimental Animals 19
2.2 Optic Fibre Implant Construction for In Vivo Optogenetics 20
2.3 Stereotaxic Surgeries for Optic Fibre Placement 20
2.4 Open Field Experiments 22
2.4.1 Experiment Set-Up 22
2.4.2 Optogenetic Stimulation Paradigm 23
2.4.2.1 Open Field Day 1 and Day 2 23
2.5 Implant Placement Verification 24
2.6 Video Processing 25
2.6.1 Open Field Day 1 and 2 26
2.6.1.1 Normalized Distance Travelled 26
2.6.1.2 Locomotive Bouts 26
2.6.1.3 Non-locomotive Event Analysis 27
2.7 Statistical Analyses 27
Chapter 3. Results 29
3.1 Stimulation of lateral SNC DANs causes an increase in horizontal movement. 29
3.1.1 Stimulation of lateral SNC DANs causes a novelty-dependant increase in the rate of horizontal movement. 31
3.1.2 Stimulation of lateral SNC DANs inhibits habituation in a novelty-dependant manner. 33
3.1.3 Stimulation of lateral SNC DANs increases locomotion, but not locomotion vigour, in a novelty-dependant manner. 35
3.2 Stimulation of lateral SNC DANs causes an increase in non-locomotive exploratory behaviour that is novelty-dependant. 39
Chapter 4. Discussion 43
4.1 Phasic stimulation of SNCL DANs increases voluntary exploratory activity in a novel environment. 43
4.2 Phasic stimulation of SNCL DANs in low-novelty environment inhibits habituation. 45
4.3 Phasic stimulation of SNCL DANs increases locomotion by increasing the number of initiations of locomotion, rather than increasing locomotion vigour. 47
4.4 The Role of the lateral SNC in Modulating Voluntary Behaviour: A Potential Model 49
4.5 Future Directions 52

Chapter 5. Concluding Remarks 54
Chapter 6. Supplemental Information 55
Chapter 7. Bibliography 70

List of Figures

Figure 1. Basal Ganglia Circuitry 3

Figure 2. Fibre optic implant placement for optogenetic experiments 22

Figure 3. Stimulus paradigm for optogenetic open field experiments 25

Figure 4. Activity levels on first exposure to open field 30

Figure 5. Stimulation of DANs differentially impacts the average rate of horizontal movement depending on stimulus location and prior exposure to the open field 32

Figure 6. Stimulation of SNCL DANs differentially inhibits the habituation of horizontal activity depending on prior exposure to the open field. 34

Figure 7. Lack of habituation in SNCL-stimulated animals can be attributed to a lack of decrease in baseline activity throughout open field exposure 36

Figure 8. Stimulation of SNCL DANs differentially increases locomotion, but not locomotion vigour, depending on prior exposure to the open field 38

Figure 9. Stimulation of DAN terminals in the DLS does not positively modulate locomotion. 40

Figure 10. Stimulation of SNCL DANs differentially inhibits the habituation of locomotive initiation depending on prior exposure to the open field 42

Figure 11. Lack of habituation in SNCL-stimulated animals can be attributed to a lack of decrease in baseline locomotion throughout open field exposure 44

Figure 12. Stimulation of SNCL DANs increases non-locomotive activity in a novelty-dependant manner 46

Figure 13. Stimulation of DAN axon terminals in the DLS has no effect on non-locomotive events 48

Figure 14. Phasic stimulation of SNCL DANs promotes exploratory behaviour specifically in a novel environment and interferes with habituation. 50

Supplementary Figure 1. Examples of optic fibre tracks 55

Supplementary Figure 2. Proposed model of the effect of phasic stimulation over time 56

List of Tables

Table S1. Statistical Analyses related to Figures 5-13. 57

List of Abbreviations

ADHD	Attention deficit hyperactivity disorder
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
BG	Basal Ganglia
DAN	Dopaminergic Neuron
DAT	Dopamine Active Transporter
D1R	Type 1 Dopamine Receptor
D2R	Type 2 Dopamine Receptor
DS	Dorsal Striatum
DLS	Lateral Dorsal Striatum
DMS	Medial Dorsal Striatum
GABA	Gamma(γ)-Aminobutyric acid
GPi	Globus Pallidus Internal Segment
I_H	Hyperpolarization-activated cyclic nucleotide-gated current
HCN	Hyperpolarization-activated cyclic nucleotide-gated channel
LTD	Long-term Depression
LTP	Long-term Potentiation
MSN	Medium Spiny Neuron
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate Receptor
OCD	Obsessive Compulsive Disorder
OF	Open Field
PD	Parkinson’s Disease
RPE	Reward Prediction Error
RRF	Retrorubral Field
SNC	Substantia Nigra Pars Compacta
SNR	Substantia Nigra Pars Reticulata
SNCL	Lateral Substantia Nigra Pars Compacta
SNCM	Medial Substantia Nigra Pars Compacta
STN	Subthalamic Nucleus
TH	Tyrosine Hydroxylase
VTA	Ventral Tegmental A

Acknowledgements

I would firstly like to extend a very heartfelt thank you to my supervisor, Dr. Raad Nashmi. I have worked and volunteered in the Nashmi lab for 5 years as of the completion of this thesis, and the volume of knowledge I have gained regarding all aspects of scientific endeavor, from experiment design to mentorship, is too vast for this section. Thank you for your patience, your passion for uncovering the mysteries of the midbrain, and for your support throughout this and other projects. I will always be immensely proud to share that I have been a member of this lab.

Thank you also to Dr. Kerry Delaney, for your wry humor and clear-eyed guidance as I "chased the ghost". Your mentorship has helped shape who I have become as an academic, for the better, and as a person, maybe for the worse. I treasure it greatly.

I would also like to thank Dr. Craig Brown, for helping me to see the bigger picture in my research and for holding my work to a high standard.

Thank you to the undergraduate students who have volunteered or otherwise contributed many, many hours in support of this research, in alphabetical order: Adeeb A., Amran A., Edoarda F., Fynn L., Katherine H., Olivia W., Jacob F., and Sydney P.

To the Coffee Commiserating Club of the Second Floor, also in alphabetical order, thank you for your support and friendship as we wrestle through the highs and lows of research together: Alberto D., Christopher A., Farnoosh F., Foad A., Laura H., Taylor K., and Tracy M.

And last, but not least, to Keyrian, for getting me hooked on neurons 6 years ago and for your love and camaraderie ever since.

Chapter 1. Introduction

1.1 Background and Rationale

For decades scientists have investigated how dopaminergic signalling in the basal ganglia is involved in voluntary behaviours. This endeavor has been complicated by the fact that basal ganglia dopamine and their dysfunction are involved in many physiological and pathophysiological processes, and consequently isolating circuit functionality can be difficult. There is a precedent in the scientific literature for transient bursts of nigrostriatal dopamine to precede, and cause, voluntary movement. In addition, there is evidence that suggests the nigrostriatal pathway (dopamine neurons of the substantia nigra pars compacta projecting to the dorsal striatum), are functionally segregated along the mediolateral axis. However, no study has yet attempted to isolate either nigrostriatal pathway's involvement in voluntary movement. Furthermore, studies probing the role of phasic dopamine in causing movement typically restrict their analyses to limited aspects of movement, such as paw movement or locomotion, and limit their observations to sec and sub-sec intervals surrounding stimulus onset. The objective of this study is to examine the role of phasic dopaminergic signalling along the lateral nigrostriatal pathway in voluntary movement by examining behaviour over longer time periods (min vs sub-sec), which allows the incorporation of a greater range of exploratory and non-exploratory behaviours into the analysis.

1.2 The Basal Ganglia: An Overview

Despite decades of study, the role of the basal ganglia (BG) in motor processing and execution remains unclear. The canonical view of the BG is that it is a system of subcortical nuclei that function to permit the smooth execution of voluntary movements and inhibit unwanted movements (Albin *et al*., 1989; Graybiel *et al*., 2000). Early discovery of the BG and their role in movement was facilitated by the discovery that BG pathology led to a number of movement-related disorders, including Parkinson's and Huntington's disease (Pirker *et al*., 2002; Cepeda *et al*., 2014). In addition to movement, the BG are critical for the learning and contextualization of behaviours and

emotional regulation (reviewed in Packard and Knowlton, 2002; Graybiel, 2005; Goldberg *et al*., 2013; Pierce and Péron, 2020; Rusu and Pennartz, 2020).

It is thought that the BG permits wanted movements by acting as a coincidence detector that inhibits the activity of motor cortical output until there is integration of sufficient permissive signals. Chronic inhibition is supplied by the BG's output nuclei, which influence the cortex by way of the thalamus. The output nuclei are comprised of the internal segment of the globus pallidus (GPi), the substantia nigra pars reticulata (SNR), and the ventral pallidum. The major input center of the basal ganglia is the striatum, comprising the caudate and putamen in primates. It receives and integrates signals from the cortex, amygdala, thalamus, cerebellum, ventral tegmental area (VTA), and the substantia nigra pars compacta (SNC), and functions as a putative "gate-keeper" of voluntary movement (reviewed in Alexander and Crutcher, 1990; Lanciego *et al*., 2012; Goldberg *et al*., 2013; Mattell and Meck, 2004; Guo *et al*., 2015). The SNC projects predominantly to the striatum, but also to the cortex directly. A more in-depth review of the basal ganglia and their role in movement can be found in the following reviews by: Alexander (1990, 1994), Dudman and Krakauer (2016), and Klaus and colleagues (2019). The focus of this thesis is a component of the BG called the nigrostriatal pathway.

1.2.1 The Direct and Indirect pathway

The idea that BG operate through largely parallel circuits is not a new concept (Alexander *et al.,* 1986; Gerfen 1988; Alexander and Crutcher, 1990). The most well-known example is the direct and indirect pathway, which function in parallel to promote wanted and inhibit unwanted movements, respectively. Both pathways are required for the proper execution of voluntary movement (Cui *et al.,* 2013; Friend and Kravitz, 2014; Klaus *et al*., 2017).

The striatum is composed of roughly 90% GABAergic medium spiny neurons (MSNs), which project to the BG output nuclei, and 10% cholinergic and GABAergic interneurons that modulate MSN activity (Gerfen, 1988; Kawaguchi *et al*., 1995, 1997; English *et al*., 2012). MSNs that receive signals from the cortex, thalamus, and the dopaminergic neurons of the SNC project to the BG output nuclei either through a monosynaptic (direct) or polysynaptic (indirect) pathway. Whether an MSN is part of the direct or indirect pathway can be characterized by its dopamine receptor expression. MSNs that express dopamine receptor D1 (D1R) are part of the direct pathway

and promote movement by inhibiting the GPi and SNr, thereby disinhibiting the thalamus. MSNs that express the dopamine receptor D2 (D2R) are part of the indirect pathway and inhibit the GPe, which releases inhibition of the subthalamic nucleus (STN). The STN sends excitatory projections to the GPi and SNr, thereby promoting inhibition of the thalamus and the downstream motor cortex (Alexander *et al*., 1986).

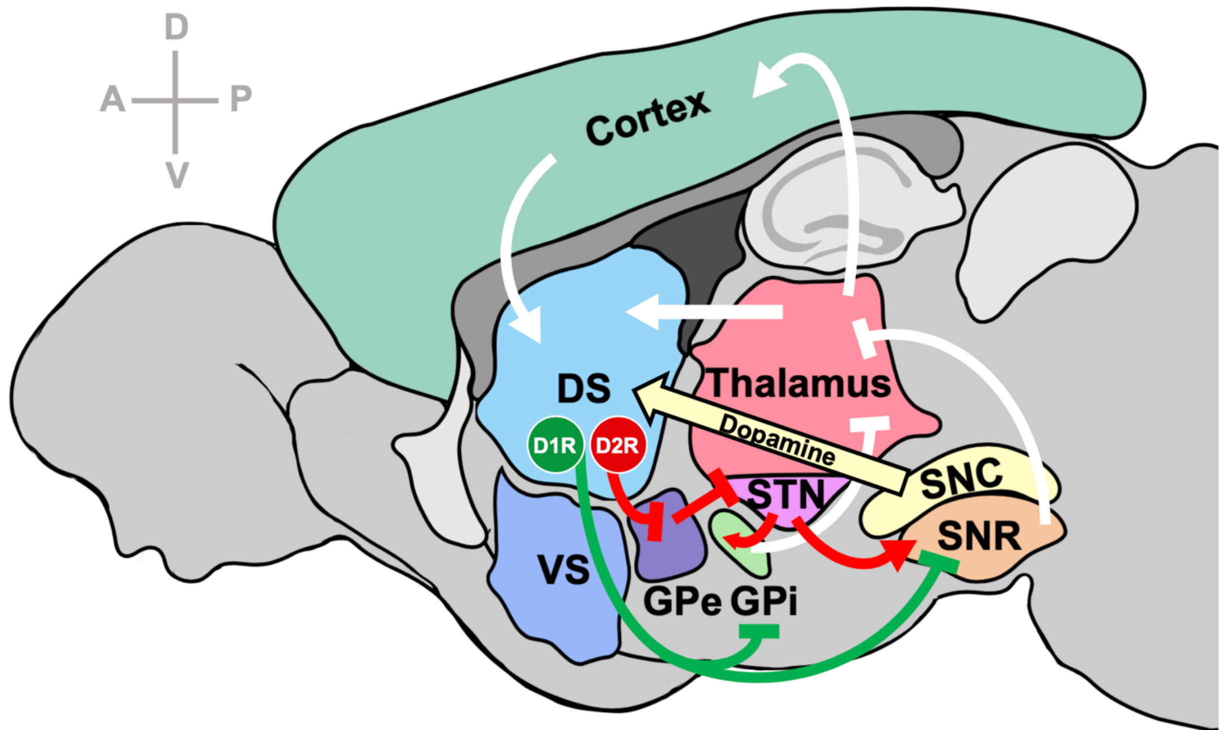


Figure 1. Basal Ganglia Circuitry. Graphic depicts sagittal view of mouse brain. The SNC sends dopaminergic projections (yellow) to the dorsal striatum. Non-yellow arrows: glutamatergic projections; clubs: GABAergic projections. Green clubs depict MSN (medium spiny neuron) projections making up the direct pathway; red clubs and arrows depict projections making up the indirect pathway. DS: dorsal striatum; VS: ventral striatum. GPe: external segment of the globus pallidus; GPi: internal segment of the globus pallidus. SNC: substantia nigra pars compacta; SNR: substantia nigra pars reticulata; STN: subthalamic nucleus.

1.2.1.1 Dopamine Receptors

D1Rs and D2Rs are G protein-coupled receptors that activate and inhibit adenylyl cyclase (AC), respectively (Stoof and Kebabian, 1981) and are powerful modulators of the strength of glutamatergic synapses on MSNs (Hersch *et al*., 1995; Smith *et al.*, 1998). D1R-mediated activation of AC leads to the activation of protein kinase A (PKA) and the resulting phosphorylation of a number of downstream intracellular targets, including the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR) (Snyder *et al*., 2000) and N-methyl-D-aspartate (NMDAR) glutamate receptors (Hallett *et al*., 2006). D1R-mediated phosphorylation of glutamate receptor subunits potentiates glutamatergic currents by increasing receptor insertion into dendritic spines, the location of glutamatergic synapses (Snyder *et al*., 1998; Hallet *et al*., 2006; Tukey *et al*., 2013). In addition, D1R activation potentiates MSN response to glutamatergic currents by promoting their transition into "up-states", *i.e.,* a depolarized membrane potential from which an action potential spike can be generated from (Galarraga *et al*., 1997; Cepeda *et al*., 1998). This is thought to be due in part to the enhanced opening of L-type Ca^{2+} channels (Surmeier *et al*., 1995, 2007) and NMDARs (Cepeda *et al*., 1993; Levine *et al*., 1996; Snyder *et al*., 1998; Flores-Hernandez *et al*., 2002). Interestingly, recent work suggests that D1R activation can extend the upstate of MSNs associated with sensorimotor and associative corticostriatal loops, which are related to movement execution, and shorten the upstate of MSNs associated with mood and motivation (Prager *et al*., 2020; Graybiel and Matsushima, 2020). The net effect of D1R activation appears to be to increase direct-pathway, *i.e.,* striatonigral, MSN excitability following sustained, coordinated glutamate signalling and inhibit MSN excitability in response to brief, uncoordinated glutamate signalling (Surmeier *et al.,* 2007).

D2Rs have the opposite effect of D1Rs and serve to decrease the excitability of MSNs of the indirect, or striatopallidal, pathway, even if the MSNs are in an "up-state" membrane potential (Hernandez-Lopez *et al.,* 2000). This is achieved through a number of mechanisms, including D2R coupling to G protein-activated inwardly rectifying potassium channels (GIRKs), and coupling to G protein complexes that decrease adenylyl cyclase activity (Stoof *et al.,* 1981; Jin *et al*., 2013). Activation of D2Rs has also been shown to decrease AMPAR-mediated currents (Cepeda *et al.,* 1993) and increase withdrawal of AMPARs from the cell membrane (Håkansson *et al.,* 2006; Surmeier *et al*., 2007).

It is tempting to silo D1Rs as purely excitatory and D2Rs as purely inhibitory in their effect on MSN responsiveness, with the intuitive extension that D1Rs contribute to long-term potentiation (LTP) (Kerr and Wickens, 2001; Anwyl, 2009), and D2Rs contribute to long-term-depression (LTD) (Kreitzer and Malenka, 2007) of synapses. However, there is evidence that both D1Rs and D2Rs contribute to Hebbian bidirectional plasticity in striatal MSNs (Shen *et al*., 2008). In support of segregating their functionality, there is evidence that suggests targeting the direct pathway through optogenetic stimulation of D1R can alleviate Parkinsonian motor symptoms of freezing and decreased ambulation in a mouse model, whereas targeting the indirect pathway through D2R stimulation has the opposite effect (Kravitz *et al.,* 2010). It is clear that there is still much work to be done to relate how dopamine affects MSN excitability and synapse strength and how this translates into behavioral phenotypes.

1.2.2 The Dorsal Striatum

The striatum is a large and complex brain region that receives inputs from the thalamus, brainstem, and topographical inputs from the cortex that further divide it into functional domains. The ventral striatum forms connections with limbic cortical regions, the dorsolateral striatum (DLS) with sensorimotor cortical regions, and the dorsomedial striatum (DMS) with associative and prefrontal cortical regions (Lynd-Balta and Haber, 1994; Reep *et al*., 2003; Graybiel, 2008). The ventral striatum, also known as the nucleus accumbens, receives projections from the midbrain dopaminergic ventral tegmental area (VTA) and has long been recognized as being integral to encoding motivation, reward, acquiring motivated behaviours, and in reinforcement learning (Apicella *et al*., 1991; Hernandez *et al*., 2002; Atallah *et al*., 2007; Graybiel, 2008, Graybiel and Grafton, 2015). The dorsal striatum (DS) is the major target of the SNC and plays an integral role in the execution of motor behaviours, including action execution (Bailey and Mair, 2006: Martinez *et al*., 2022), action selection (Bissonette and Roesch, 2015), and habit development (reviewed in Graybiel 2008, Graybiel and Grafton, 2015).

As mentioned above, the DS can be divided into medial and lateral subregions that form corticostriatal loops with associative and sensorimotor cortical regions respectively. The DMS is critical for learning and executing motivated behaviours that are affected by the anticipated outcome of the behaviour. Once a behaviour is repeated sufficiently with a positive outcome,

neural activity shifts to the DLS, which is critical for the development and execution of behaviours that are no longer sensitive to behaviour outcome. For example, habitual motor skills that have motor-motor associations and do not require explicit memory or stimulus to be executed, such as locomotion, are encoded by the DLS. With repetition of an action, DLS activity becomes more pronounced at the beginning and end of the action sequence, with the inverse occurring in the DMS (Thorn *et al*., 2010; reviewed in Graybiel and Grafton, 2015). This DLS pattern of activity has been termed "task bracketing" or "chunking", and was proposed by Graybiel and Grafton (2015) to be the brain's way of protecting successful behaviours from degradation. This chunking of activity in the DLS suggests that behaviour is a concatenation of behavioral units, and indeed this theory is supported in recent reports investigating dopaminergic signalling in the DLS (Markowitz *et al*., 2018; 2023). With its functional and topographical segregation, the DLS is situated to be a major effector of changes in movement-related signalling, and a major target of changes in signalling from the upstream dopaminergic midbrain.

1.2.3 The Dopaminergic Midbrain

Despite its relatively small size, the importance of the dopaminergic midbrain in normal functioning is evidenced by the large number of pathologies that result from its dysfunction, including Parkinson's disease (Przedborski, 2005), attention-deficit hyperactivity disorder (ADHD) (Leo *et al*., 2003; Jucaite *et al*., 2005), obsessive-compulsive disorder (OCD) (Xue *et al*., 2022), addiction (Pidoplichko *et al.*, 1997), and depression (Chaudhury *et al*., 2013). There are three dopaminergic nuclei in the ventral midbrain: the ventral tegmental area (VTA), the substantia nigra pars compacta (SNC), and the retrorubral field (RRF), all of which project to the striatum and supply the BG with dopamine. The VTA and SNC are located largely within the same rostral-caudal planes, whereas the RRF is situated more dorsal and caudal (Jimenez-Castellanos and Graybiel, 1987; German and Manaye, 1993). Based on projection target, dopamine neurons (DANs) can be divided into mesostriatal, mesolimbic, and mesocortical populations.

A large body of evidence supports the theory that within which subregion a dopamine neuron (DAN) resides has a large impact on the information that it encodes and the behaviour that its activity is associated with. DANs of the VTA have been shown to have a role in reinforcement learning (Solié *et al*., 2022), encoding of reward (Keiflin *et al*., 2019), novelty (Morrens *et al*.,

2020), working memory (Levin *et al.,* 1994), decision making (Stopper *et al*., 2014), motivation (Nishino *et al*., 1987), and aversion (Brischoux *et al*., 2009). SNC DANs also have a strong role in reinforcement learning (Diao *et al*., 2021) and are involved in the encoding of reward (Schultz *et al*., 1993), novelty (Morrens *et al*., 2020), stimulus valence (Lerner *et al*., 2015), goal-directed learning (Jin and Costa, 2010), and voluntary movement (Jin and Costa, 2010 Panigrahi *et al*., 2015; Howe and Dombeck, 2016; Da Silva *et al*., 2018). RRF DANs may play a role in aversion signalling (Moaddab and McDannald, 2021). This functional topography lends itself to the idea that the BG operate in parallel information streams.

1.3 The Substantia Nigra Pars Compacta

1.3.1 Dopamine Neurons of the Substantia Nigra Pars Compacta: An Overview

DANs, as their name suggests, synthesize and release the neurotransmitter dopamine. DANs are often characterized by their expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis that is correlated with DAN survival with age (Chu *et al.,* 2002). In addition, DANs are characterized through their expression of the dopamine transporter (DAT), which regulates re-uptake of dopamine by the presynaptic neuron (Carboni *et al.,* 1990; Sonders *et al*., 1997). SNC DANs express high levels of nicotinic receptors and are powerfully modulated by nicotine (Grenhoff *et al*., 1986; reviewed in Nashmi *et al*., 2006, 2007). Endogenously, they receive cholinergic input from the pedunculopontine nucleus (PPN) and the laterodorsal tegmental nucleus (LDT), which are located in the brainstem (Xiao *et al.*, 2016; Estakhr *et al.,* 2017). In addition to cholinergic input, they are adjacent to and inhibited by the GABAergic SNR. Roughly 30% of all presynaptic terminals forming synapses with DANs are glutamatergic ($VGluT2^{+}$) and 40-70% are GABAergic (Henny *et al.*, 2012).

DANs exhibit either a fusiform or multipolar cell body shape (Liang *et al*., 1996), express D2Rs pre and postsynaptically (Centonze *et al*., 2002), and release dopamine from their soma and dendrites. Consequently, they can autoinhibit and modulate afferent input strength with local dopamine release (Paladini *et al.,* 2003; Hikima *et al*., 2021). In addition, their axons typically arise from a primary dendrite rather than the soma, which allows back-propagating action potentials that may contribute to DAN pacemaking activity (Gentet and Williams, 2007).

1.3.2 SNC Dopamine Neuron Firing Characteristics

DANs have broad action potentials and slow, autonomous pacemaking activity that arises from slowly depolarizing pacemaker potentials mediated by hyperpolarization activated cyclic nucleotide gated (HCN) channels (Chu and Zhen, 2010). This tonic, single-spike, slow firing at typically less than 8Hz and is thought to facilitate greater release of local dopamine and contribute to self-regulation of firing rate (Grace and Bunney, 1984a; Estakhr *et al*., 2017).

DANs also fire in high frequency bursts (<500ms, 12-20Hz), although this burst activity is not cell-autonomous and requires glutamatergic input (reviewed in Paladini and Roeper, 2014). Burst firing typically consists of trains of 3 to 10 action potential spikes that begin with an inter-spike interval of 80ms (12.5Hz) that increases throughout the burst to 160ms (6.25Hz) or longer, followed by periods of inactivity (300ms) (Grace and Bunney, 1984b). The shift from tonic to burst firing occurs in response to unexpected, salient environmental cues and precedes voluntary movement (reviewed in Paladini and Roeper, 2014). The detailed firing properties of DANs are outside the scope of this thesis and can be found described in detail elsewhere (see Palidini and Roeper, 2014; Gantz *et al*., 2018).

1.3.3 Dopaminergic Neuronal Heterogeneity Across Different Spatial Regions of the SNC

As mentioned earlier, there is a substantial body of evidence that suggests that the DANs of the ventral midbrain are a heterogenous population (see section 1.2.3). Midbrain nuclei can be separated based on projection targets, inputs, firing characteristics, protein expression, morphology, and vulnerability to degeneration. There is also a large body of evidence that suggests that within the SNC DANs are heterogenous along the dorsoventral, rostrocaudal, and mediolateral axes (Prensa and Parent, 2001; Neuhoff *et al*., 2002; Murase and McKay, 2006; Evans *et al*., 2017; Fu *et al*., 2012; Estakhr *et al*., 2017; reviewed in Gantz *et al*., 2018). Of particular relevance to the work in this study is the difference in DANs along the mediolateral axes. Schiemann and colleagues (2012) found that K-ATP channel expression in medial SNC (SNCM) DANs, but not lateral, are required for novelty-dependant exploration. In the rat midbrain the lateral SNC (SNCL) expresses less TH and DAT mRNA and less TH protein than the SNCM (Blanchard *et al*., 1994). Earlier work in this lab has demonstrated that the SNCM receives primarily cholinergic-mediated

GABAergic currents that decrease locomotion in mice when cholinergic terminals are activated, whereas the SNCL receives cholinergic-mediated excitatory currents that increase locomotion when cholinergic terminals are stimulated. Furthermore, SNCL DANs have a more fusiform shape and larger I_H currents, whereas SNCM DANs have a more multipolar morphology, smaller I_H currents, a more hyperpolarized membrane potential, and lower average tonic firing frequency (Estakhr *et al.*, 2017). In the 2015 study by Lerner and colleagues, it was demonstrated that the medial and lateral SNC DANs form parallel, reciprocally connected nigrostriatal pathways with the medial and lateral dorsal striatum, respectively, and that these pathways encode different responses to aversive stimuli. SNCL DANs were positively modulated by aversive stimuli, whereas SNCM DANs were negatively modulated, suggesting that these different subpopulations encode saliency and the valence of a stimulation differently. These results support findings by Matsumoto and Hikosaka (2009), who observed that dorsolaterally located DANs in monkey midbrains were excited by aversive stimuli whereas ventromedially located DANs were inhibited by the aversive stimuli. Based on this generous amount of evidence, it is not unreasonable to hypothesize that the medial and lateral SNC form distinct populations with distinct roles in behaviour.

1.4 Substantia Nigral Dopamine and Movement

Despite decades of research following the discovery that the death or dysfunction of dopaminergic neurons in the SNC causes a range of movement disorders, the role of dopamine in movement remains unravelled and controversial. This is likely due to a couple of interacting factors, namely the heterogeneity of SNC DANs, the observation that DAN activity both precedes and is modulated by movement, and the observation that DAN activity is also correlated with sensory stimuli in a context-dependant manner. Although there is considerable evidence that tonic release of dopamine is required for permitting and even promoting movement (Spielewoy *et al.,* 2000), the focus of this thesis is on the phasic release of dopamine and how it contributes to the selection and execution of voluntary movement.

1.4.1 The Reward Prediction Error Hypothesis

A well-known function of ventral midbrain DANs in both the VTA and SNC is their role in encoding whether a stimulus is rewarding, or more specifically, reinforcing. The seminal work of Schultz (1998, 2002) made a strong case for reward being a main driver of the nigrostriatal system. It is believed that DANs fire phasically in response to unexpected reward, thereby linking a given action to a rewarding outcome. In addition, phasic dopamine signalling encodes the value of a given reward, with rewards of greater size and probability of occurring being valued more (Fiorillo *et al*., 2003; Tobler *et al*., 2005; Hamid *et al.,* 2016). Taken together with the observation that modulations to dopaminergic signalling occur after an action has been planned and executed (Howe and Dombeck, 2016; Da Silva *et al*., 2018), these findings would suggest that dopamine is required for adapting ongoing behaviour to suit a given context.

Context is an important element in dopaminergic signalling. As Barter *et al*., (2015) explain, in critique of behavioural studies that restrain animals, behaviour is not the result of simply processing sensory input to generate motor output. Instead, behaviour is "the result of a comparison between input and reference". Phasic DAN firing is thought to act as that "comparison" device, by encoding a reward prediction error (RPE), *i.e.,* the difference between what occurs and what was expected. The animal then putatively adapts its behaviour to minimize this difference (Schultz, 2007; reviewed in Graybiel and Grafton, 2015). RPE encoding has been demonstrated in a number of studies and animal models, including humans, examples of which can be found in the reviews by Lerner and colleagues (2021) and Diederen and Fletcher (2021). This would suggest that phasic DAN firing helps animals learn what actions result in an appealing stimulus, or reward, and thereby select behaviours that maximize return.

However, there is increasing evidence that reward isn't always necessary for phasic DAN activity to modulate behaviour. As discussed in the following section, there is a growing body of evidence that suggests phasic DAN activity precedes and is modulated by voluntary movements that are not driven by an extrinsic reward. This suggests that DAN signalling plays a more active role in modulating voluntary movements beyond gating and selecting for rewarding behaviours.

1.4.2 Phasic Dopamine Encodes Elements of Movement

1.4.2.1 The Role of Dopamine in Movement Onset

There is a precedent in research surrounding the dorsal striatum (DS) that striatal activity occurs at the onset and offset of well-learned behaviours (Jin and Costa, 2010; reviewed in Graybiel and Grafton, 2015). A number of studies suggest that nigrostriatal dopamine signalling may play a part in the encoding of this action initiation and termination. Jin and Costa (2010) found phasic MSN and SNC DAN activity correlated with the initiation and termination of self-paced lever-pressing sequences that mice learned to perform for a sucrose reward, and disruption of activity impaired the mice's ability to both learn and execute the sequence. Specifically, SNC DANs were found to phasically increase firing before lever pressing.

Similarly, Panigrahi and colleagues (2015) found a positive increase in extracellular striatal activity predominantly at the onset of fore-limb movement in subregions that form motor corticostriatal connections, and that this modulation is substantially reduced in a mouse model of Parkinson's disease (PD) (MitoPark Mice, Ekstrand *et al*., 2007). PD model animals exhibit behavioral deficits and have less than 20% normal dopamine levels in the striatum. Howe and Dombeck (2016) established that there is a large population of DLS-projecting DANs that display rapid and phasic signalling in response to ongoing accelerations. Da Silva and colleagues (2018) also observed that a large number of SNC DANs increase phasic activity before and during voluntary movement, specifically locomotion. These findings are part of a collection that make a case for the theory that phasic dopamine signalling can promote and even drive movement.

With the aid of optogenetics a number of laboratories have started to tackle this theory. Howe and Dombeck (2016) observed that head-fixed mice would consistently transition from rest to locomotion on a treadmill following stimulation of DAN axon terminals in the DLS; with accelerations that lasted 0.5-2 secs. Similarly, work by our lab demonstrated that in the lateral SNC, cholinergic terminals synapse on DANs and evoke EPSCs, and that optogenetic stimulation of these terminals promotes movement in freely moving animals (Estakhr *et al.,* 2017). These findings suggest at a role in phasic DAN activity in driving movement. However, a more prevalent finding that is not necessarily mutually exclusive with the former, is that DAN stimulation increases the probability of movement. Da Silva and colleagues (2018) observed that stimulation

of SNC DAN cell bodies in freely moving mice increased the vigour and probability of future movements. Hamilos and colleagues (2021) likewise were not able to directly trigger licking in a reward-driven experiment, but DAN stimulation did early-shift self-timed licking, whereas inhibition had the opposite effect. There are a number of reasons which could contribute to these mixed results, including variable study design, stimulus paradigms, and stereotaxic targets. It is clear that the question on how exactly DAN activity promotes movement remains to be answered.

1.4.2.2 The Role of Dopamine in Movement Vigour

A hallmark symptom of PD is slower, less vigorous movement (bradykinesia) (Berardelli *et al*., 2001). Striatal dopamine levels have been shown to be required for normal modulation of effort, or vigour, during voluntary actions. In the study mentioned above by Panigrahi and colleagues (2015), it was observed that the greatest modulation of dorsal striatum activity occurred early in forelimb movement execution, suggesting a role in modulating vigour. In Zénon and colleagues' 2016 study healthy humans given levodopa, a precursor in dopamine synthesis, used more force in time-constrained tasks than placebo-treated studies. Bartholomew and colleagues (2016) optogenetically stimulated the dorsal striatum of transgenic mice expressing channerhodopsin-2 (ChR2) in D1R-expressing neurons and demonstrated that pulse width and stimulus frequency are positively related to movement speed in freely moving animals. Da Silva and colleagues (2018) observed that DAN activity 300ms before action initiation was related to the vigour of future movements and that roughly 40% of recorded neurons had significantly higher activity before more vigorous movements. These finding suggests that changes in dopamine concentration during ongoing behaviour modulates the kinematics and effort of movement (Yttri and Dudman, 2016; Bova *et al*., 2020).

A recent study by Markowitz and colleagues (2023) suggests that the influence of dopamine on movement effort may have to do with the time over which the signal is integrated. The authors observed a negative relationship between endogenous dopamine transients in the DLS and forelimb movement velocity over timescales of less than 10 secs. However, this relationship switched to a positive relationship over longer timescales of 20 to 60 secs. In addition, the authors found optogenetic stimulation during high velocity movements was correlated with an increased vigour of later occurrences of that same movement. However, it should be noted that the authors

also observed that optogenetic stimulation during less vigorous movements correlated with less vigour in later occurrences of that same movement. This finding seems to point to a role of dopamine in action selection, or action quality selection, and refinement, as opposed to always driving a certain action quality (more vigorous) (see following section 1.4.2.3). Howard and colleagues (2017) similarly found that NMDA-dependant endogenous and optogenetic stimulation of nigrostriatal dopamine signalling is integral in action selection. These recent findings demonstrate that although there is a strong case for dopamine playing a role in modulating movement effort, the theory is part of a very current and ongoing discussion in the scientific community.

1.4.2.3 Role of Dopamine in Movement Refinement

In the earlier section 1.4.1 the RPE theory was discussed, in which dopamine transients are posited to function as a tool that detects salient stimuli and teaches the organism to both associate outcome with behaviour and to modulate behaviour to maximize returns. This teaching, or sculpting, of movement and behaviour in nigrostriatal circuits may not be restricted to external reward-driven scenarios. Dopamine transients have been observed after movement unrelated to reward, which would suggest a broader role in refining movement. Although Da Silva and colleagues (2018) found that most DANs were positively modulated prior to movement initiation, 28% of DANs were negatively modulated after movement onset. In contrast, Howe and Dombeck (2016) found that positively modulated DAN activity lagged behind acceleration bursts during continuous locomotion. In their recent paper, Markowitz and colleagues (2023) observed that DLS dopamine fluctuations reinforced behaviour “syllables” over minutes in freely moving mice, which coincided with positive changes in dopamine concentration. Syllables were defined as repeatable, sub-sec behaviours identified by machine learning. The authors discuss a model in which mice structure their spontaneous behaviour to maximize DLS dopamine concentrations. In this theory, endogenous fluctuations of dopamine might act as an intrinsic reward that sculpts and promotes certain behaviours. This line of reasoning, and the more general idea of dopamine as a behaviour refinement tool, might help explain why phasic stimulation of nigrostriatal pathways has been observed to increase the likelihood of future behaviours more than drive movement directly. It is

possible that the time scale over which dopamine is acting, and over which behaviour is being measured, might also be a factor.

1.5 Functional Segregation of the Lateral Nigrostriatal Pathway

There is considerable evidence that suggests that the basal ganglia operate in partially closed circuits that allow different functions to be executed simultaneously (Alexander *et al*., 1986; McHaffie *et al*., 2005). Recent work suggests that the nigrostriatal pathway may be another layer of functional segregation, with the medial and lateral pathways carrying different information. This was suggested by evidence that the SNC and DS are functionally heterogenous along the medial and lateral axes (see section 1.2.2 and 1.3.3), but is given more weight by Lerner and Deisseroth's 2015 study that demonstrates that these regions are reciprocally connected and functionally separate. Using viral tracers, they defined distinct subpopulations of DMS-projecting and DLS-projecting SNC DANs with different responses to aversive stimuli. Taking these findings into account with recent findings by our lab that stimulation of cholinergic terminals on SNCL DANs promotes locomotion, and the abundance of evidence suggesting that the DLS is integral in the selection and execution of well-known behaviours (section 1.3.3), I suspect that the lateral nigrostriatal pathway forms a functional unit between the midbrain and striatal components, with each sharing motor or behavioral roles.

1.6 Dopamine Signalling and the Novel Environment

1.6.1 Novelty Encoding

There is substantial evidence that DAN activity is stimulated by novel stimuli and helps to shape the behaviour in response to this stimulus (reviewed in Redgrave and Gurney, 2006; Redgrave *et al*., 2008, Krebs *et al*., 2011; Menegas *et al*., 2017). How dopaminergic signalling accomplishes this is not yet clear. There is a theory that novel stimuli are intrinsically rewarding, and that phasic dopamine encodes a "novelty bonus" that encourages exploration (Kakade and Dayan, 2002; Hazy *et al*., 2010). There is also evidence that dopamine plays a role in the attribution of value to novel stimuli (Costa *et al.,* 2014). Another possibility, which is not necessarily mutually exclusive, is

that DAN signalling in response to a novel stimulus may help the animal identify which elements of context or behaviour caused the unpredicted event (Redgrave and Gurney, 2006) and help the animal associate an unexpected outcome with the preceding action (Thorndike, 1911). Redgrave and Gurney (2006) also posit that phasic DAN signalling reinforces the selection of the action or behaviour that immediately precedes the unexpected, salient event, in a theory thematically similar with the findings and conclusions drawn by Markowitz and colleagues (2023), in which dopamine transients in the DLS reinforced coincident sub-sec behaviour.

It is undeniable that there is strong precedent that dopamine is involved in encoding novelty and likely plays an important role in contextualizing and reacting appropriately to novel stimuli. There is also evidence that dopaminergic signalling plays a role in the seeking out of novel stimuli.

1.6.2 Novelty Seeking

Novelty seeking can be described as the tendency of an animal to explore an unfamiliar stimuli or environment (Wilson and Goldman-Rakic, 1994*;* Reed *et al*., 1996*;* Costa *et al*., 2014) and is a necessary behaviour in order for the animal to acquire information for optimal decision making. There is evidence to suggest that DAN activity modulates novelty-seeking behaviour, as dopaminergic signalling has been correlated with novelty-seeking behaviours in humans and animals (Bardo *et al*., 1996*;* Bódi *et al*., 2009*;* Leach *et al*., 2022*).* Costa and colleagues (2014) found that administration of a DAT inhibitor, to prevent normal reuptake of dopamine in the striatum, increased preference for novel choices during a decision-making task in monkeys, and that this was due to increased value placed on the novel option. Mice with only one functional DAT gene, and consequently reduced dopamine reuptake, have been shown to have lower anxiety and explore novel environments and objects more than wild-type mice. However, mice with the DAT gene knocked out completely (KO) demonstrate greater activity than either heterozygous or wild-type animals, but with less exploration of novel objects. The KO mice also displayed greater thigmotaxis, or wall-hugging, which is a measure of anxiety (Pogorelov *et al*., 2005). Anxiety and novelty-seeking are tightly connected behaviours with a typically inverse relationship (reviewed in Pawlak *et al*., 2008). These findings suggest that more dopaminergic tone may not necessarily mean more exploration, and that there may be an ideal striatal concentration.

There is also evidence that phasic DAN activity may play a role in driving exploratory behaviour. In 2012 Scheimann and colleagues demonstrated that K-ATP channel-mediated burst firing of SNC DANs was required for novelty-dependant exploration. Silencing of K-ATP channels decreased locomotion and rearing relative to controls on the first exposure to an open field, but not on consecutive days, which suggests an interaction between burst firing, novelty, and voluntary movement. Schindler and colleagues (2017) also observed that mild traumatic brain injury-induced increases in rat exploration were accompanied by larger dopamine transients in the ventral striatum. Together these results suggest that much like with "pure" movement, dopamine is modulated by and is a likely modulator of novelty encoding and novelty-related behaviours.

1.7 The Open Field

1.7.1 An Overview

The open field (OF) test was first introduced in the 1930s as a measure of timidity in mice (Hall, 1934). Since then, it has grown in usage to become one of the most ubiquitously used behavioural assays for measuring exploratory behaviour and general activity in mice and rats (Walsh and Cummins, 1976). Generally, the OF consists of a square, rectangle, or circular enclosure, can range in size from 25cm x 25cm to over 250cm x 250cm, is made of wood, plastic, or metal, and has walls roughly 35cm high to prevent escape. OF experiments initially were quite short, only 2-10 min, to aid manual counting of behaviours. However, with the advent of video recording and tracking software, OF experiments can be considerably longer. Common measures of activity are distance moved, time spent moving, rearing, time spent in the center, and change in activity over time. It should be noted that activity levels can be affected by a number of internal factors (age, sex, motor output, exploratory drive, fear or anxiety, illness, circadian rhythm), and external factors (noise, light levels, handling, housing) (Gould *et al*., 2009). This is noteworthy, as often the purpose behind using OFs to measure voluntary behaviour is to divorce activity from external cues, such that movement can be interpreted as the result of "purely" internal cues. Instead, the OF should itself be considered a multifactorial variable that interacts with the test variable of interest (Walsh and Cummins, 1976).

1.7.2 Habituation

There are consistent patterns of behaviour that most mice display when introduced to an OF. Mice are typically very active shortly after introduction to the OF (Gould *et al*., 2009). How much the mice are initially active is affected by how stimulated, versus how frightened, they are as a result of being removed from their home cage, the unfamiliar environment itself, and whether they have any previous experience with the OF. Early activity is also the result of the novel environment eliciting an orientation reaction in the mice as they create a spatial representation of their new environment in their hippocampi (Walsh and Cummins, 1976; O'Keefe, 1990). Mice then typically exhibit habituation: a gradual decrease in exploratory behaviour as the hippocampal "map" is completed and no biologically salient result is associated with the stimulus of the new environment. Typically, habituation occurs within 30 min, after which activity level stabilizes (Walsh and Cummins, 1976*;* Gould *et al*., 2009*).* Habituation can be measured within a test day (intrasession) and between consecutive test days (intersession). The former is considered a measure of adaptation and non-associative learning, whereas the latter is considered a measure of memory retention (Leussis and Bolivar, 2006). In other words, not only is the OF a method of measuring activity levels, but it is also a method of measuring habituation and memory.

1.7.3. Dopamine and Habituation

There is evidence to suggest dopamine signalling plays a role in habituation. Homozygous DAT KO and knock-down animals have been observed to have reduced intrasession habituation to a novel environment (Spielewoy *et al.,* 2000). Mice lacking D1Rs or D1R activity also lack intrasession habituation (Giménez-Llort *et al*., 1997). In their 1997 study Giménez-Llort and colleagues suggest that dopaminergic signalling influences motor activity specifically when the animals are still in their exploratory phase prior to habituation, as dopamine receptor antagonists only modulated movement in the first hour of the OF and not the second hour. This theory is supported by the findings of Schiemann and colleagues (2012), where decreased burst firing only affected locomotion and rearing during the first 20 min of OF exposure. The question that arises from these results is: does dopamine modulation actually inhibit the adaptation and associative learning required for habituation, or is it countering habituation with increased motor stimulation? Furthermore, does phasic dopamine

signalling only impact movement in the context of a novel environment? There is a possibility that a population of SNCM DANs project to the hippocampus, where spatial memory is stored (Tsetsenis *et al*., 2021; see section 1.7.2), however there is much more evidence of midbrain dopaminergic input coming from the VTA (Broussard *et al*., 2012, 2016). Finally, to our knowledge, there has been no effort to link dopamine signalling to intersession habituation. The question whether phasic DAN signalling in the SNC affects intrasession and intersession habituation would benefit from more investigation.

1.8 Research Objectives and Hypothesis

*The **objective** of this study is to use a transgenic mouse model and optogenetics combined with open field behavioral experiments to investigate whether phasic stimulation of lateral nigrostriatal dopaminergic neuron somata and axon activation play a role in modulating voluntary movement.* We have selected the lateral nigrostriatal pathway partially for logistical reasons, as it is the easiest of the SNC subregions to target, but also because the majority of evidence points to the lateral SNC and lateral DS as being closer to modulating motor output directly than the medial nigrostriatal pathway. The aim of this study is to expand the definition of voluntary movement past the usual limit of locomotion to also include other voluntary behaviours such as supported and unsupported rearing and grooming. In addition, our aim is to include measurements of behaviour over time periods that not only allow the measurement of naturalistic behaviours, but also allow incorporation and consideration of the intersection of dopaminergic signalling with novelty. *I **hypothesize** that phasic stimulation of somata in the lateral SNC and axons in the lateral DS will increase exploratory movements during the recording period in a novelty-dependant manner.*

Chapter 2. Materials and Methods

2.1 Experimental Animals

All experimental procedures were conducted in accordance with guidelines set by the Canadian Council for Animal Care (CCAC) and by those in an animal care protocol approved by the Animal Care Committee at the University of Victoria. Mice were group housed with up to 5 animals per cage prior to surgeries and experiments. After stereotaxic surgeries mice were singly housed to limit damage to fibre optic implants. Mice were housed on a 12-hour light-dark cycle in which lights were turned on at 7am and turned off at 7pm. Mice were given food and water *ad libitum*. The mice used in experiments were between 2-4 months old.

The mouse strains used for the experiments in the study were *DATcre* and *DATcre::ChR2(H134R)-EYFP*. The *DATcre* transgene was heterozygous (het) for both mouse lines while the *ChR2(H134R)-EYFP* transgene was homozygous (hom) for the *DATcre::ChR2(H134R)-EYFP* mouse line. In both strains cre recombinase expression is driven by the dopamine transporter (DAT) promoter. The purpose of using mice het for *DATcre* as opposed to hom is because hom *DATcre* knock-in mice have been shown to have ~50% reduction in normal DAT expression, with behavioral differences from het and wild-type (wt) animals, while het mice have been shown to have a minimal decrease in DAT expression (Giros, 1996; Bäckman *et al*., 2006).

The *ChR2-EYFP* gene contains an upstream *loxP*-flanked STOP cassette. Consequently, *ChR2-EYFP* expression is conditionally expressed only in cre-recombinase expressing cells. In the *DATcre(het)::ChR2(H134R)-EYFP(hom)* strain, only DAT-positive, *i.e.,* dopaminergic cells, express cre recombinase and therefore express ChR2-EYFP.

The *DATcre(het)* line was produced by crossing hom *DATcre* (Bäckman *et al*., 2006) (JAX, stock# 006660) knock-in mice with C57BL/6J (JAX, stock# 000664) mice. The *DATcre(het)::ChR2(H134R)-EYFP(hom)* line was produced by crossing the hom *ChR2-EYFP* mouse line (Ai32(RCL-ChR2(H134R)/EYFP) on a C57BL/6J background (Madisen *et al*., 2012) (JAX, strain# 024109) with hom *DATcre* mice. The resulting *DATcre(het)::ChR2(H134R)-EYFP(het)* offspring were bred until sufficient levels of *DATcre(het)::ChR2(H134R)-EYFP(hom)* offspring were produced for use in experiments and as breeders. These breeders produce a mix of *DATcre(hom)::ChR2(H134R)-EYFP(hom),* *DATcre(wt)::ChR2(H134R)-EYFP(hom),* and

DATcre(het)::ChR2(H134R)-EYFP(hom). The former two strains were used as parental strains that when bred reliably produced the desired experimental strain, *DATcre(het)::ChR2(H134R)-EYFP(hom)*. For the ease of the reader, the *DATcre(het)* strain will here on out be referred to as opsin-negative animals, and the *DATcre(het)::ChR2(H134R)-EYFP(hom)* strain will be referred to as opsin-positive or opsin-expressing.

2.2 Optic Fibre Implant Construction for In Vivo Optogenetics

Implantable optic fibres were crafted from 0.5NA 200µm core multimode fibre (FP200URT, ThorLabs). Roughly 4cm of the fibre was stripped with a 235-280µm cladding, 457-533µm coating range fibre stripping tool (T12S21, ThorLabs). The stripped fibre was separated from the coating-covered fibre by scoring the stripped fibre with a ruby fibre scribe (S90R, ThorLabs) and pulling as described by Ung and Arenkiel (2012). The stripped fibre was inserted into either a 10.5mm (CFX270-10, ThorLabs) or 6.4mm-long (CFLC230-10, ThorLabs) ceramic ferrule. Two ferrule lengths were used as it aided assembly of the dental cement cap during surgery, especially for the midbrain targets. Either 4.5mm or 3mm of stripped fibre were left exposed, depending on the targeted brain region. The ferrule and fibre optic were secured together with epoxy (F112, ThorLabs) and cured with a heat gun. Both ends of the fibre optic were then polished by holding the implant perpendicular to polishing paper and making repeated eight-shaped rotations. Four consecutive grades of polishing paper were used: 5, 3, 1, and 0.3µm (LF5P, LF3P, LF1P, and LF0.3P, ThorLabs). Polished implants were then attached to the same LED used in optogenetic behaviour experiments (M455F3, ThorLabs) and their optical output measured with a power meter (PM400, ThorLabs). The minimum acceptable output was set to 3mW (Da Silva *et al.,* 2018). Implants with an optical output of less than 3mW were re-polished until they met this criteria. Implant output ranged between 3-7mW.

2.3 Stereotaxic Surgeries for Optic Fibre Placement

Adult animals (2-4 months) were anesthetized with 2% inhaled isoflurane in an induction chamber. After the animals reached surgical depth of anesthesia, confirmed with a lack of toe pinch reflex, the animals were maintained at 1-2% isoflurane for the duration of surgery. Once at surgical depth

of anesthesia, the mouse was placed onto a stereotaxic frame (SKU# 51615, Stoelting Co.) and its head stabilized with ear bars. Tear gel was applied at surgery onset and re-applied throughout the duration of the surgery to prevent damage to the corneas. A rectal thermometer attached to a warming pad was used to prevent the animal's temperature from dipping below, or exceeding, an acceptable physiological range. The surgical area over the skull was cleaned with 70% ethanol and 100µL of lidocaine was injected under the scalp. While the lidocaine was absorbed the cleaned area was shaved. A midline incision was made in the scalp to expose the skull, and the skull surface was cleaned and dried with a sterile Q-tip. The animals were given 100µL of sterile saline every thirty min throughout the surgery to prevent dehydration.

The mouse head was ensured to be level by measuring the skull height at bregma, lambda, and the regions where the medial and lateral burr holes were to be drilled. A bone anchor screw (SKU 51462, Stoelting Co.) was inserted into the skull anterior of bregma and posterior of lambda to ensure the dental cement cap would not be pried off by the mouse. Effort was made to keep the location of the support screws consistent across all animals.

Two holes were drilled into the skull at the following coordinates relative to bregma, based on Franklin and Paxinos' stereotaxic atlas (2008) and the Mouse Brain Atlas online tool developed by Dr. Matt Gaidica (http://labs.gaidi.ca/mouse-brain-atlas/): lateral SNC: -3.0 to -3.2mm AP, +/- 1.2 to 1.3mm ML; lateral DS: +0.5 to -0.1mm AP, +/- 2.4 to 2.5mm ML. The optic fibre implant was attached to a stereotaxic cannula holder (SCH 1.25, Doric Lenses) and the reference height of the skull measured just medial of the burr hole. The pial surface of the brain was nicked with a sterile needle to ensure that the pia mater did not impede the optic fibre implant. The implant was then slowly lowered into the desired position (lateral SNC, -4mm from skull surface, lateral DS, -2.7 to -2.8mm) and fixed in place with opaque dental cement (SKU S380, C&B Metabond®) (Figure 2A). The process was repeated for the second implant. As the resulting dental cement cap was too large to allow re-suturing of the wound, the area was left open, and the animals monitored over two consecutive days for signs of discomfort and infection. After the dental cement had dried completely, the animals were put in a clean warmed cage with access to wet food and water and monitored until they were walking normally.

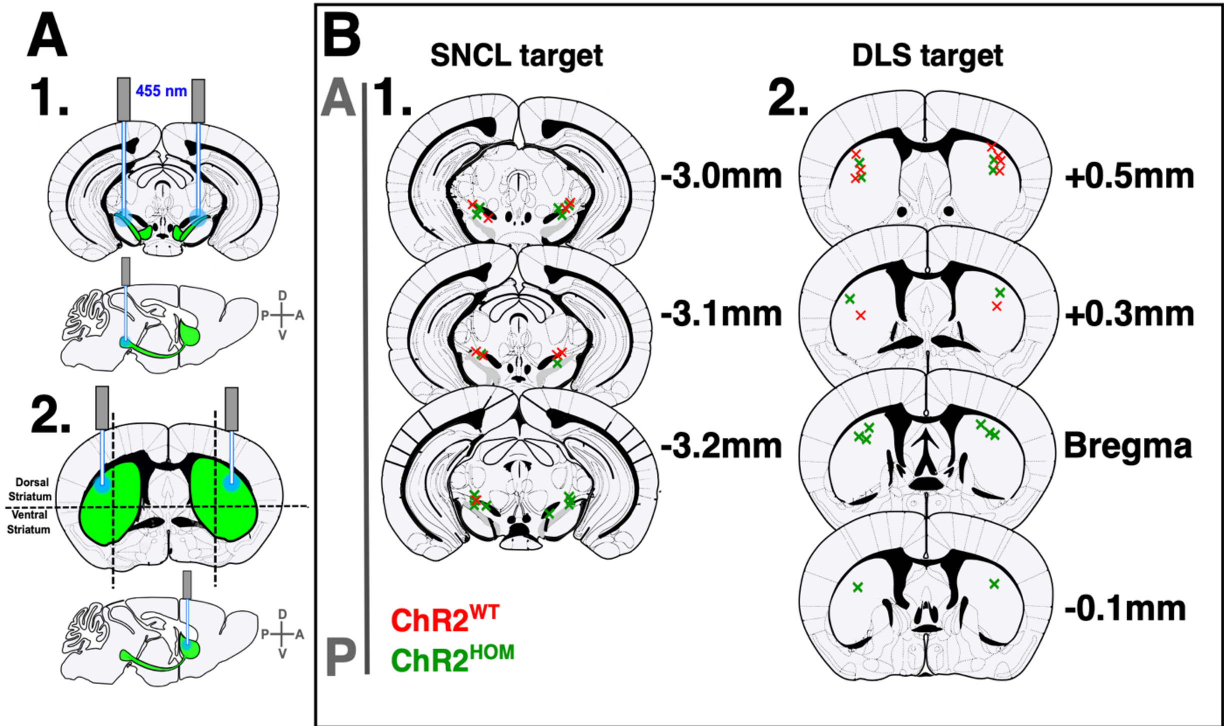


Figure 2. Fibre optic implant placement for optogenetic experiments. A. Target optic fibre implant placement for the lateral substantia nigra pars compacta (SNCL, 1.) and the lateral dorsal striatum (DLS, 2.) Green indicates ChR2-YFP expression in regions containing dopaminergic cell bodies (1.) and axons (2.). **B.** Actual fibre optic implant placement in the SNCL and DLS. Red crosses indicate animals not expressing ChR2-YFP, and green crosses indicate animals positive for ChR2-YFP.

2.4 Open Field Experiments

2.4.1 Experiment Set-Up

After 1 week of recovery from surgery, mice were brought in their home cage into the OF procedure room and given at least 20 min to acclimatize to the room in ambient light while the stimulation and recording apparatus was set-up by the experimenter (Figure 3A). To deliver blue light to the implants, a pair of 200µm mono-fibre optic patch cords (MFP_200/230/900-0.57_1.5m_SMA-ZF1.25, Doric Lenses) were attached to one 455nm LED each (M455F3, ThorLabs). A 455nm LED was chosen for optogenetic experiments as 450nm is the peak absorption spectra of ChR2(H134R) (Lin, 2011). Before each experiment, including between

animals, the fibre-LED power output was checked using a power meter (PM400, ThorLabs). If the power output was less than 11mW the mono-fibre optic patch cord was replaced.

After their acclimatization period mice were weighed and immobilized with 2% isoflurane delivered with an induction chamber. The mono-fibre optic patch cords were attached to the ceramic ferrules of the implants with ceramic split mating sleeves (ADAL1-5, ThorLabs) and a sheath fixed over the implants to contain escaped LED light. The mice were then allowed to recover for a minimum of 10 min, or until walking normally. On average, it took roughly 5 min to immobilize the mice and attach the mating sleeves to the implant ferrules. Control animals that did not receive implant surgeries were immobilized with isoflurane for 5 min and given a minimum of 10 min to recover.

Once the mice were walking normally, they were introduced to a corner of the OF and their activities recorded with a video camera (Sony Digital HD video camera recorder, HDR-SR1, Handycam) with a collection frequency of 30fps. The open field used was 40cm(l) x 18cm(w) x 30cm(h) and lined with black construction paper on the outside and inside walls to prevent the mice from seeing the experimenter during trials and to minimize reflections from the implants during LED stimulation. A red LED that received the same input from the pulse driver as the LEDs driving the optic fibre implants was taped to the inside of the upper wall of the OF to aid the experimenter in visualizing when the LED was on during later video processing (Figure 3A). The OF was wiped down with 70% ethanol between mice and the paper replaced regularly to avoid scent build-up. Experiments were performed between 10am and 3pm and the order of mice (opsin-negative vs opsin-positive) alternated to avoid one group always starting before the other. During the experimental trials, the LED was delivered at a maximum of 1.5A and the pulses were triggered with a Neuro Data PG4000 Digital Lab Stimulator. After the OF experiment, mice were again briefly immobilized with 2% isoflurane to remove the mono-fibre optic patch cord and mating sleeve and returned to their home cage after they had begun to walk normally again. Controls lacking implants received the same treatment.

2.4.2 Optogenetic Stimulation Paradigm

2.4.2.1 Open Field Day 1 and Day 2

For each experiment day, 4 consecutive 9 min videos were recorded for a total period in the OF of approximately 40 min. The stimulus paradigm of each of the videos was identical. No stimulation was given for the first 3 min in order to establish a baseline of activity. The baseline was followed by a 3 min trial period and a 3 min stimulus-free recovery period. The 3 min trial period was broken up into 3 x 30-sec periods of stimulation with 30 secs of recovery. Each 30 sec stimulation period contained 15 repetitions of the LED being on for 1 sec and off for 1 sec. The LED was flashed in 10ms pulses at 12.5Hz (80ms inter-pulse interval) (Figure 3B).

2.5 Implant Placement Verification

After animals had completed all behaviour experiments, they were intracardially perfused with 20mL of 1M PBS (4mL/min) followed by 25mL of 4% paraformaldehyde (PFA) (4mL/min). The brains were removed and fixed overnight in 4% PFA at 4°C, then stored in 1M PBS at 4°C until sectioning. The brains were cut into 100µm sections on a vibratome (Pelco 101 Vibratome, Series 1000) and stored in 1M PBS at 4°C until mounting. Sections were mounted with Immu-Mount (9990402, Epredia, Fisher Scientific) with the pH adjusted to 7.9-8.0 using 5M NaOH. After allowing the Immu-Mount to harden overnight at 4°C, sections were imaged on a Nikon C1si laser scanning confocal inverted microscope (Eclipse Ti-E, Nikon) using a 4X air CF1 Plan Apo VC objective (0.12NA, 17.2mm working distance), a digital camera (SKU A3550U, OMAX), and the ToupView digital camera control software (ToupTek Photonics). At least one implant termination point was identified per brain section before a given animal could be considered for analysis (Figure 2B). No animals were excluded from analysis.

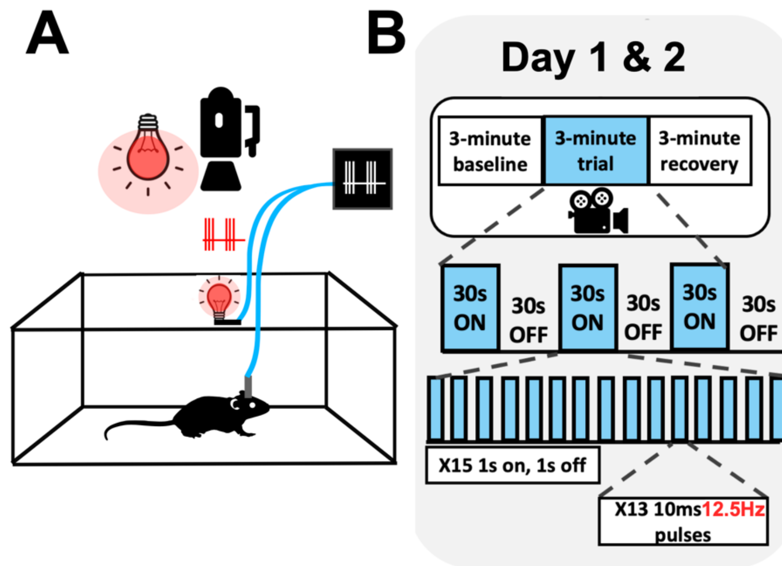


Figure 3. Stimulus paradigm for optogenetic open field experiments. **A.** Schematic of open field stimulation and recording apparatus. A pulse train is simultaneously delivered to both the optic fibre implants and a red LED situated near the top edge of the open field. **B.** Stimulus protocol for open field experiments. During the trial period a 12.5Hz (80ms inter-pulse interval) pulse train is delivered in 1 sec on, 1 sec off intervals repeated within 30-sec stimulus blocks.

2.6 Video Processing

Using a video editor (Wondershare Filmora, version 10.5.3) each 9 min video (MPEG file) was separated into 3 min subsections (baseline, trial period, recovery). To determine when each section began, the first frame in which either the red LED flashed, or blue light could be seen around the implant cap/sheath, was considered the start of the trial period and used as the reference time point. In instances where both red and blue light were visible at different time points, whichever light occurred earlier was used as the reference. The 3 min video sections were exported as MP4 files and converted into AVI files using the file conversion software FFmpeg (Tomar, 2006).

The AVI files were imported into the video processing program Fiji (Schindelin *et al.*, 2012) and every third frame selected for further processing. Each frame was smoothed with a Gaussian filter (sigma 10) and a manual threshold run to separate foreground from background (0-48 foreground pixel values). To obtain the position of the animal, the Fiji plugin Analyze Particle was used with animal-sized particles defined as being 400mm-Infinity and having a circularity of 0.1-1.0. The Analyze Particle settings were adjusted until the plugin outputted one value for each

frame. To check the accuracy of the plugin to separate the mouse from dark areas in the OF, the position coordinates were plotted as a path in the statistical computing and graphics environment R Studio (R Core Team, 2020). In instances where the path had sharp peaks indicative of mistaken particle inclusion, the experimenter converted the position coordinates into distance travelled between frames and plotted distance versus frame to identify the problematic frame(s). The experimenter then erased the mistaken particles manually, re-adjusted the Analyze-particle settings, and repeated until the path was smooth. The distance travelled and related values for the baseline, trial, and recovery periods were concatenated into one file, exported from R Studio as a CSV file, then converted into an Excel file.

2.6.1 Open Field Day 1 and 2

2.6.1.1 Normalized Distance Travelled

The Excel file was imported into the data analysis environment IgorPro (IgorPro8, version 8.04, 64-bit, WaveMetrics) and the distance values smoothened using a median filter (5 points). The integral of the distance x time wave was measured for the 3 min baseline, the 3 x 30 sec periods when the LED was on (3-3.5 min, 4-4.5 min, 5-5.5 min), and the 3 min recovery. To normalize, the sum distance was divided by the number of min it occurred over, 3 min or 90 secs/1.5 min.

2.6.1.2 Locomotive Bouts

The Excel spreadsheet with distance and time variables outputted at the end of section 2.6 was separated into a spreadsheet that contained the last 90 sec of baseline (1.5-3 min), a separate spreadsheet for each 30 sec LED on period on (3-3.5 min, 4-4.5 min, 5-5.5 min), and the last 90 sec of recovery (7.5-9 min). Each of these was run through a custom R Studio script that defined locomotive bouts as rows containing velocity values at or above 5cm/s for at least 300ms (Ruder *et al*., 2016; Da Silva *et al*., 2018). The number of bouts, mean bout velocity, mean bout length, and distance travelled throughout all of the bouts was outputted and saved.

2.6.1.3 Non-locomotive Event Analysis

Non-locomotive events were counted manually throughout the 3 min baseline, trial, and recovery video segments. Almost all counts were done by an experimenter blinded to the experimental group of the animals. Non-locomotive events were defined as the following:

Supported rearing: Mouse raises up on its hind legs and places one or both paws on the walls of the OF (Crusio *et al.,* 1986; Sturman *et al.,* 2018).

Unsupported rearing: Mouse raises up on its hind legs without touching the walls of the OF (Sturman *et al.,* 2018).

Grooming: Mouse scratches with its hind legs or cleans itself with its forepaws (Crusio *et al.,* 1986).

Escape behaviours: Mouse clings to the OF wall or attempts to jump out of the OF. These were very uncommon and consequently were not analyzed on their own, but rather included in sum measures of activity.

2.7 Statistical Analyses

Values are expressed as mean ± standard error. Statistics were analyzed using the R Studio statistical analysis environment (R Core Team, 2020). In all cases where both time interval and experimental group (opsin-negative vs opsin-positive) were included in analysis, a two-way mixed repeated analysis of the variance (ANOVA) was run to determine if there was a significant main effect in any of the two factors of LED stimulation, mouse genotype, and their interaction. If there was a significant difference in the main effects, then this was followed by post-hoc pair-wise t-tests with Bonferoni correction. In cases where multiple time intervals were compared within a single group, a one-way repeated ANOVA was used for parametric data and a Friedman Rank Sum test was used for nonparametric data. Data were determined to be parametric or not by testing for normalcy with the Shapiro-Wilk test and for homogeneity of variance with the Fligner-Killeen test. Post-hoc analyses were conducted if the mixed repeated ANOVA, one-way repeated ANOVA, or Friedman Rank Sum test indicated a statistically significant ($p < 0.05$) effect of genotype, time interval (LED stimulation), or their interaction. Post-hoc analyses used were

Student's t-test with a Bonferroni correction for parametric data or Wilcoxon rank-sum test with a Bonferroni correction for nonparametric data. Data were only considered statistically significant at $p < 0.05$. Effect size and power calculations between two means were done in R Studio using the cohen.d and pwr.t.test functions. In all cases n represents number of mice.

Chapter 3. Results

3.1 Stimulation of lateral SNC DANs causes an increase in horizontal movement.

We stimulated ChR2-YFP-expressing (opsin-positive) DAN somata in the SNCL and axon terminals in the DLS with a protocol designed to mimic the firing frequency at the onset of phasic, *i.e.,* “burst”, firing in DANs (12.5Hz, Grace and Bunney, 1984; Jin and Costa 2010) (Figure 3B). We recorded over 3 min segments as previous published and unpublished work by our lab, and others investigating cholinergic inputs into the SNC, found similar multi-min time lengths effective for measuring gross changes in activity levels (Xiao *et al*., 2016; Estakhr *et al.,* 2017). Furthermore, measuring over consecutive periods allowed us to examine changes in activity related to exploration of an OF environment as its novelty declines.

In order to get a sense of over what time frame within these 3 min segments the stimulation could be causing a change in activity levels, we created histograms of activity “events” for the first OF experiment day (OF Day 1) (Figure 4). Events were defined as any movement value that met or exceeded 1cm in a frame (0.1s). Histogram bins were set to 6 sec, or 0.1 min, and the event counts normalized to the number of animals in each group.

When the normalized counts per 0.1 min are averaged across the four videos, a distinctive pattern of activity appears for the opsin-expressing group with SNCL optic fibre implants (Figure 4Ai). During the 3 min trial period (min 3-6) there is a distinct increase in activity relative to baseline levels, and the increase appears to be greatest within the 30 sec LED-ON periods. The increase in activity also appears to be sustained, and bleeds over into the 3 min recovery period. This trend is not observed in the opsin-negative SNCL implant groups (Figure 4Aii), or in the DLS implant (Figure 4Bi,ii) and no implant groups (Figure 4Ci,ii). This could be an indication that stimulation of DAN axon terminals in the DLS failed to cause a change in movement. However, another possibility is that potentially subtle changes are not being captured by this fairly stringent thresholding of activity (0.1cm/0.1s, *i.e*., 10cm/s). We concluded that these histograms suggest that our paradigm mimicking phasic stimulation is effective, at the very least in the SNCL, at causing an increase in activity that is time-locked to LED onset.

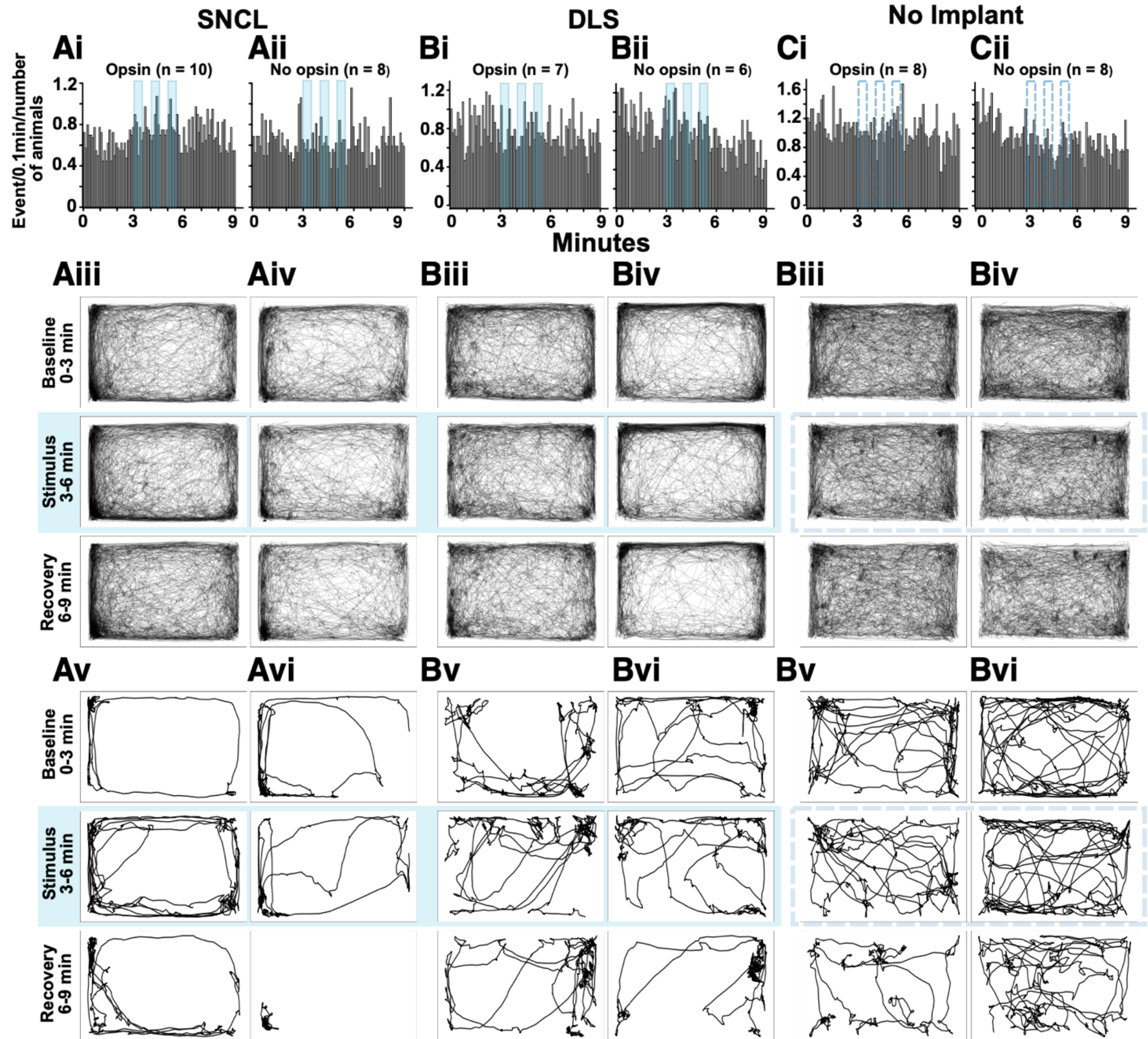


Figure 4. Activity levels on first exposure to open field. Histogram bins are 0.1 min. Filled blue bars represent LED delivery to animals with implants. Empty blue bars represent the LED pulse train being delivery to an external red LED only. **Ai.** Opsin-expressing animals with implants over the SNCL (n = 10) show a time-locked increase in activity (averaged across 4 consecutive videos) at LED onset relative to baseline (min 0-3) activity levels, which persists into the recovery phase (min 6-9). This is not replicated in opsin-negative animals with SNCL implants (n = 8) **(Aii)**. Time-locked increase in activity is not replicated in opsin-positive (n = 7) **(Bi)** or negative (n = 6) **(Bii)** animals with DLS optic fibre implants, or opsin-positive (n = 8) **(Ci)** or negative (n = 8) **(Cii)** animals with no implants. Traces from videos 1-4 overlaid for animals with SNCL optic fibre implants, opsin-positive (**Aiii**), opsin-negative (**Aiv**); DLS optic fibre implants, opsin-positive (**Biii**), opsin-negative (**Biv**); no optic fibre implants, opsin-positive (**Ciii**); opsin-negative (**Civ**). Example trace from a single, representative animal with SNCL optic fibre implants, opsin-positive (**Av**), opsin-negative (**Avi**); DLS optic fibre implants, opsin-positive (**Bv**), opsin-negative (**Bvi**); no implants, opsin-positive (**Cv**), opsin-negative (**Cvi**).

3.1.1 Stimulation of lateral SNC DANs causes a novelty-dependant increase in the rate of horizontal movement.

We next examined how horizontal movement is affected by stimulus when all horizontal movements are included in analysis. We repeated the stimulus paradigm across two days of OF exposure in order to see how a change in environment novelty would impact our ability to stimulate movement. In agreement with the activity histograms (Figure 4), on OF Day 1 the rate of total horizontal movement exhibited a statistically significant increase from baseline (18% increase, $p = 0.0378$) during the stimulus period for the opsin-positive SNCL implant group when averaged across videos (Figure 5Ai). Although the rate of movement did not return to baseline levels during the recovery period, the activity during recovery was not significantly different from baseline (14.0% increase from baseline, $p = 0.198$). This increase in activity during the stimulus period was not replicated in the opsin-negative SNCL implant group (9.0% decrease between baseline and trial period, $p = 0.636$) (Figure 5Ai), in either DLS implant group (opsin-positive, 19.8% decrease, $p = 0.125$; opsin-negative, 1.8% decrease, $p = 1$) (Figure 5Bi) or in either non-implanted group (opsin-positive, 17.1% decrease, $p = 0.0509$; opsin-negative, 16.4% decrease, $p = 0.053$) (Figure 5Ci). In addition, the increase in activity rate during the stimulus period was not replicated in any group tested on the second day of OF exposure (OF Day 2), which suggests an involvement of environment novelty in the ability of phasic stimulation to drive movement.

We next analyzed the rate of activity during individual 30 sec LED-ON periods in order to determine whether behavioral change was occurring throughout the stimulus period, as phasic dopaminergic signalling has been shown to induce changes in neuronal plasticity (Figure 5Aiii, Biii, Ciii) (reviewed in Reynolds and Wickens, 2002; Mastwal *et al*., 2014; Reynolds *et al*., 2022). During OF Day 1 there appears to be an upward trend in activity rate with each consecutive LED-ON period for the opsin-expressing SNCL group, but there is no statistically significant difference between intervals ($p = 0.127$) (Figure 5Aiii). However, when comparing the means of the rate of activity between the baseline and the third LED-ON period, an effect size (Cohen's d) of 0.87 (large) and a power ($1-\beta$) of 0.68 was calculated. A power of 0.68 is below what is generally accepted as the minimum level of power (≥ 0.8) and in order to be 95% (0.95) confident in the difference, an n of 19 was calculated. Likewise, for the increase in activity measured between the

averaged baseline and stimulus-period in the OF Day 1, opsin-positive SNCL group (Figure 5Ai, 18% increase, p = 0.0378) a Cohen's d value of 0.45 was calculated. This is indicative of a small

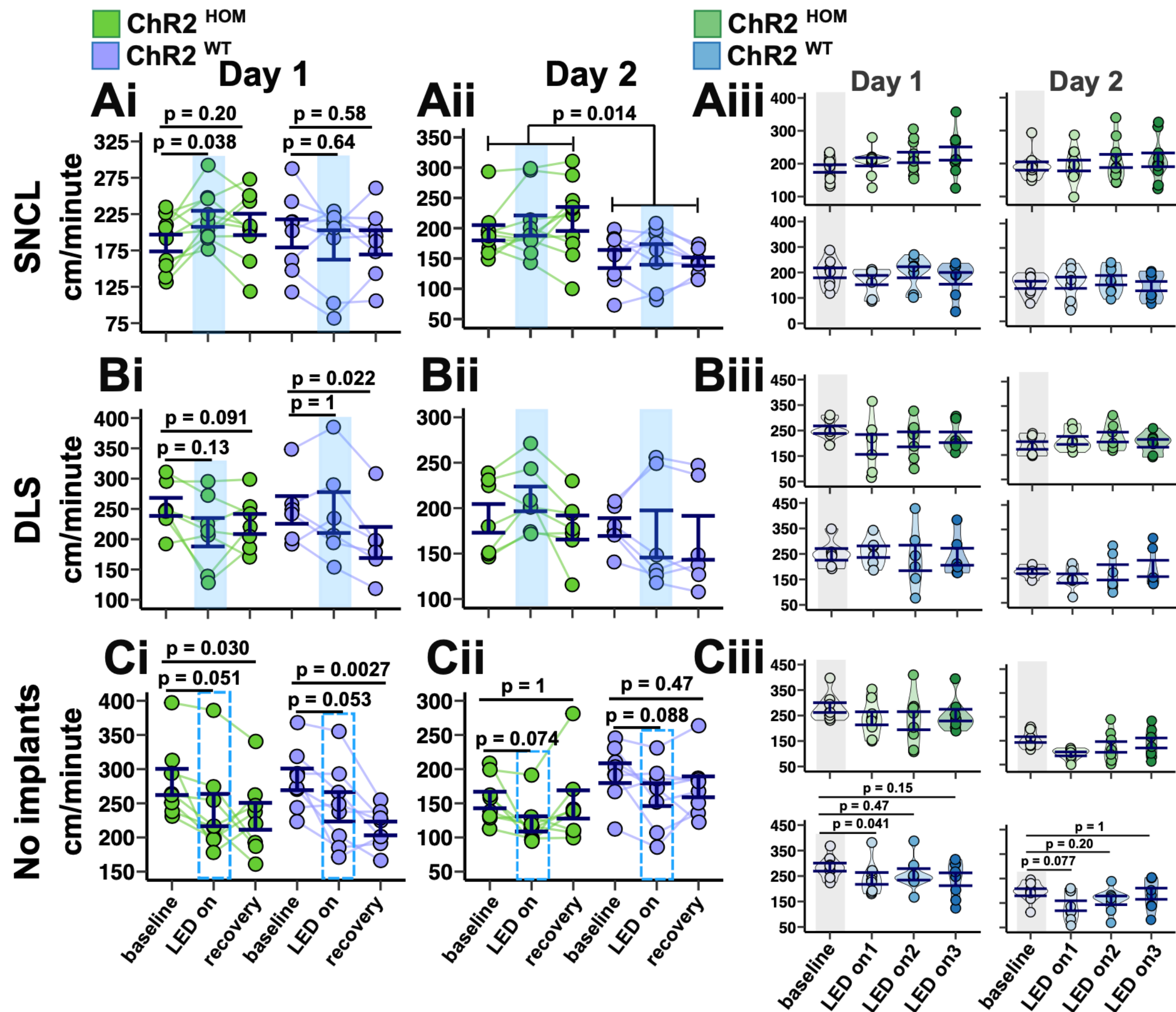


Figure 5. Stimulation of DANs differentially impacts the average rate of horizontal movement depending on stimulus location and prior exposure to the open field. Values represent normalized rates of activity, *i.e.*, total distance travelled normalized to the number of min over which the value was obtained (baseline, 3 min; stimulus period, 3x30s LED-ON periods, 1.5 min; recovery, 3 min), for each animal, averaged across 4 consecutive videos. Error-bars are the mean ± standard error of the mean. Activity rate of animals with fibre optic implants over the SNCL (opsin-positive: n = 10, 6 male, 4 female; opsin-negative: n = 8, 4 male, 4 females) during the first exposure to the OF (**Ai**) and the second exposure to the OF (**Aii**). Comparisons were made using a two-way mixed repeated ANOVA (**Ai and Aii**) followed by a post-hoc paired Student's T-test with Bonferroni correction (**Ai**). Activity rate of animals with fibre optic implants over the DLS (opsin-positive: n = 7, 3 male, 4 female; opsin-negative: n = 6, 2 male, 4 female) during the first exposure to the OF (**Bi**) and the second exposure to the OF (**Bii**). Comparisons were made with a two-way mixed repeated ANOVA (**Bi and Bii**) followed by a post-hoc paired Student's T-

test with Bonferroni correction (**Bi**). Activity rate of animals with no implants (opsin-expressing: n = 8, 4 males, 4 females; opsin-negative: n = 8, 4 males, 4 females) during the first exposure to the OF (**Ci**) and the second exposure to the OF (**Cii**). Comparisons were made with a two-way mixed repeated ANOVA and a post-hoc paired Student's T-test with Bonferroni correction (**Ci and Cii**). Comparison of normalized activity rates between baseline and individual LED-on periods for SNCL-implanted animals (**Aiii**), DLS-implanted animals (**Biii**) and non-implanted animals (**Ciii**). Day 1 comparisons: one-way repeated ANOVA for both opsin-positive group and opsin-negative group (**Aiii, Biii, Ciii**). Day 2 comparisons: one-way repeated ANOVA for opsin-positive group (**Aiii, Biii, Ciii**) and Freidman's test for opsin-negative group (**Aiii, Biii**). **Ciii.** Opsin-negative group compared with one-way repeated ANOVA.

to moderate sized effect (0.2-0.5). However, the power, given this d value, was calculated to be 0.25. This is quite low considering the 0.8 power minimum. In order to be 80% confident with the same parameters a recommended n of 41 is required. It is possible that with a greater n (>10) a statistically significant increase would be observed between the three successive LED-ON periods in the opsin-positive SNCL group (Figure 5Aiii) and in the opsin-expressing DLS group on OF Day 2 (n = 7), as a small increase was observed (Figure 5ABii, 11%, p = 0.228).

3.1.2 Stimulation of lateral SNC DANs inhibits habituation in a novelty-dependant manner.

Although there was no average increase in the rate of horizontal movement during the stimulus period relative to baseline for any group on OF Day 2, the opsin-expressing SNCL group did show an increased overall amount of activity relative to the opsin-negative group, irrespective of interval (baseline, trial, recovery) (Figure 5Aii, p = 0.0135). Two questions arise from this finding. The first, are there in fact stimulus-induced increases occurring on OF Day 2, but they are being washed-out by the averaging across videos? The second, is the separation in activity level between groups evidence of the groups habituating to the no-longer novel OF at different rates? To answer these questions, we plotted the SNCL implant animals' normalized activity rates for individual videos (Figure 6) and compared these to their baseline values (Figure 7). On OF Day 1 and Day 2 there is no individual video in which the stimulus period correlated with a significant increase in activity, although video 1 (min 0-9) on OF Day 1 almost reached significance (Figure 6Ai, p = 0.0572), as did video 1 when averaged across OF Day 1 and 2 (Figure 6Ci, p = 0.065). This suggests that the stimulus-induced increase in activity is a variable and subtle effect that requires

averaging across trials to be detected. However, it is likely that stimulation has the greatest effect on activity levels in the first ten min of exposure to the OF.

Regarding the second question, by video 4 (30-39 min in the OF) on both OF Day 1 and OF Day 2 there is a difference in overall activity levels between groups (Figure 6Aiv, Biv, Civ).

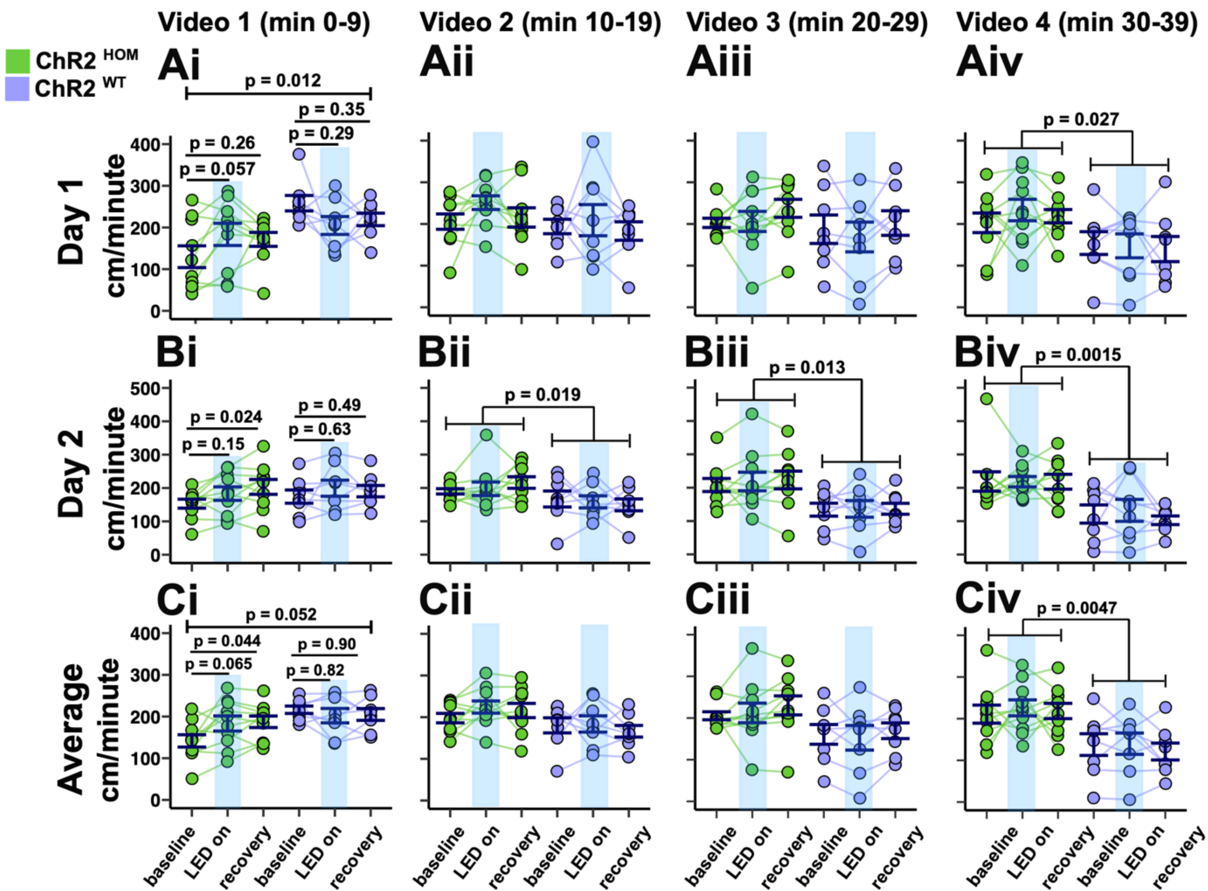


Figure 6. Stimulation of SNCL DANs differentially inhibits the habituation of horizontal activity depending on prior exposure to the open field. Values represent normalized rates of activity, *i.e.*, total distance travelled normalized to the number of min over which the value was obtained (baseline, 3 min; stimulus period, 3x30s LED-ON periods, 1.5 min; recovery, 3 min) for each animal, averaged across 4 consecutive videos. Error-bars are the mean ± standard error of the mean. Opsin-expressing animals: n = 10, 6 males, 4 females. Opsin-negative animals: n = 8, 4 males, 4 females. Activity rates during the first day of OF exposure during the first (**Ai**), second (**Aii**), third (**Aiii**), and fourth (**Aiv**) recording session. Activity rates during the second day of OF exposure during the first (**Bi**), second (**Bii**), third (**Biii**), and fourth (**Biv**) recording session. Activity rates averaged across the two days of OF exposure during the first (**Ci**), second (**Cii**), third (**Ciii**), and fourth (**Civ**) recording session. Comparisons were made with a two-way mixed repeated ANOVA followed by a post-hoc paired Student's T-test with Bonferroni correction (**Ai, Bi, Ci**).

On OF Day 2 the separation starts earlier at video 2 (10-20 min in the OF), with the separation increasing in statistical significance with each consecutive video. We reasoned that if the stimulation was causing the opsin-expressing group to habituate less than the control group to the increasingly familiar OF, then the opsin-expressing animals' baseline activity would be relatively constant, or positively modulated, across videos whereas the opsin-negative animals' baselines would steadily decrease. During OF Day 1 the opsin-negative group shows marked habituation, with a 40% decrease in baseline activity between video 1 (0-3 min) and video 4 (30-33 min) (Figure 7Ai, $p = 0.0234$). This decrease in activity is absent in the opsin-positive group. However, it should be noted that the video 4 baseline activities between groups are not significantly different (Figure 7Aii, $p = 0.195$).

On OF Day 2, there is a lack of statistically significant habituation in baseline values for the opsin-negative group (Figure 7Bi, $p = 0.213$). Interestingly, there is a 24% ($p = 0.00585$) increase between the baselines of video 1 (0-3 min) and 2 (10-13 min) for the opsin-expressing group (Figure 7Bi), that mirrors the same group on OF Day 1 (Figure 7Ai, 58% increase, $p = 0.0996$). Also of note, the video 4 baseline (30-33 min) of the opsin-expressing group was found to be 80% higher than those of the opsin-negative group ($p = 0.0291$) (Figure 7Bii). Averaged across the two OF days, there is a general trend of a gradual decrease in baseline values for the opsin-negative group and an increase in baseline values for the opsin-positive group that culminate in a significantly higher video 4 baseline activity compared with the control group (Figure 7Cii, 53% greater, $p = 0.0449$). These findings suggest that phasic stimulation of SNCL DANs impedes normal habituation to an environment of decreasing novelty.

3.1.3 Stimulation of lateral SNC DANs increases locomotion, but not locomotion vigour, in a novelty-dependant manner.

The horizontal movement captured in the above analysis encompasses a wide array of possible voluntary movements, including posture changes, head movements, walking, and sniffing. We next asked whether phasic stimulation specifically promoted locomotion, *i.e.,* the animal committing to walking or darting, and whether previous exposure to the OF affected the ability of phasic stimulation to promote locomotion. We defined locomotion as movement bouts that were

a minimum of 300ms long at velocities of 5cm/s or greater (Ruder *et al.,* 2016; Da Silva *et al.,* 2018) (Figure 8F). In agreement with our total horizontal movement findings, we observed a stimulus-dependant increase in the number of locomotive bouts (30.0% increase from baseline, p = 0.0174) and an almost significant increase in the total distance travelled during locomotion

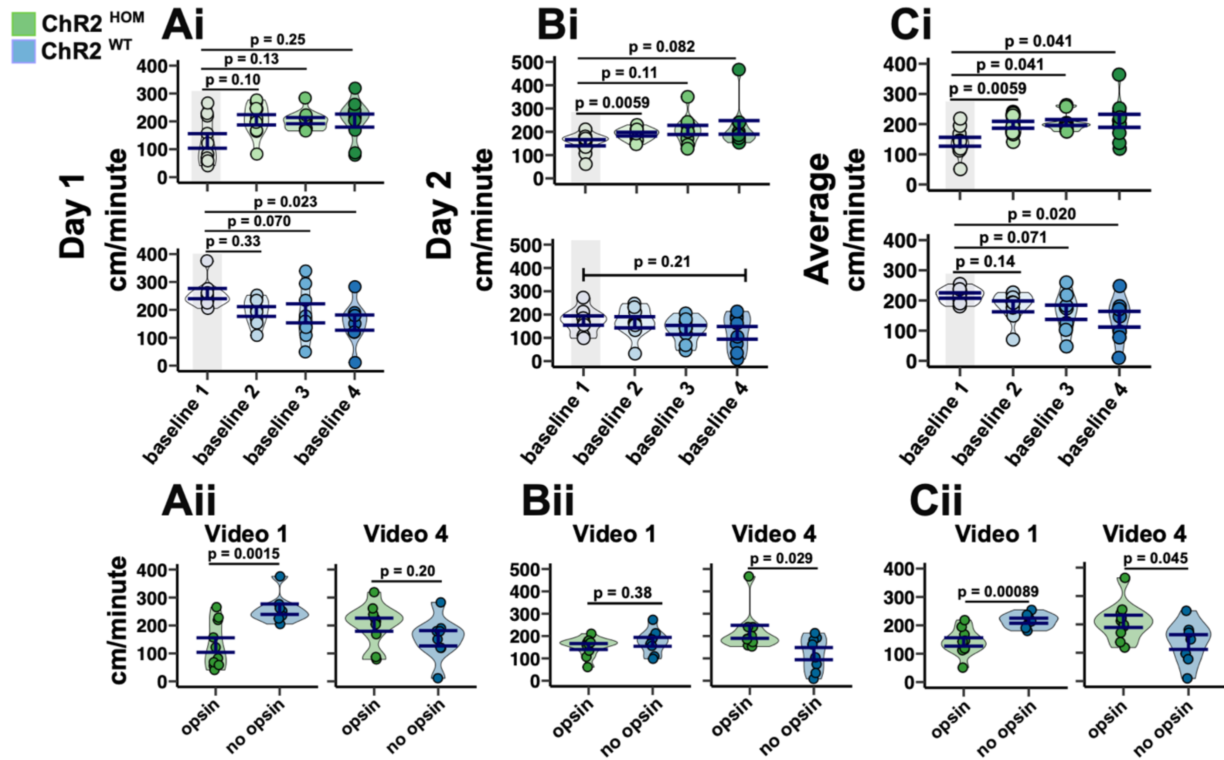


Figure 7. Lack of Habituation in SNCL-stimulated animals can be attributed to a lack of decrease in baseline activity throughout open field exposure. Values represent normalized rates of baseline activity, *i.e.*, total distance travelled normalized to the number of min over which the value was obtained (baseline, 3 min; stimulus period, 3x30s LED-ON periods, 1.5 min; recovery, 3 min) for each animal. Baseline activity occurs over minutes 0-3 (baseline 1), 10-13 (baseline 2), 20-23 (baseline 3), and 30-33 (baseline 4). Error-bars are the mean ± standard error of the mean. Opsin-expressing animals: n = 10, 6 males, 4 females. Opsin-negative animals: n = 8, 4 males, 4 females. **Ai.** Baseline activity levels across 4 consecutive recording sessions during the first exposure to the OF. Comparisons were made with a one-way repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction (opsin-positive) and a Freidman's test with a post-hoc paired Wilcoxon Rank Sum test with Bonferroni correction (opsin-negative). **Bi**. Baseline activity levels across 4 consecutive recording sessions during the second exposure to the OF. Comparisons were made with a Friedman's test with a post-hoc paired Wilcoxon test with Bonferroni correction (opsin-positive) and a one-way repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction (opsin-negative). **Ci.** Baseline activity levels across 4 consecutive recording sessions averaged across the 2 days of OF exposure. Comparisons were made with a Friedman's test with a post-hoc paired Wilcoxon test with Bonferroni correction

(opsin-positive) and a one-way repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction (opsin-negative). Comparison of the first and fourth baseline activity levels between groups during the first exposure to the OF (**Aii**), the second exposure to the OF (**Bii**) and when averaged across the 2 days of exposure (**Cii**). Comparisons between groups were done with an unpaired Student's T-test.

(27.9% increase from baseline, p = 0.069) on OF Day 1 for the opsin-expressing SNCL group that was not replicated in the opsin-negative SNCL group (Figure 8Ai, Bi). Both of these values remained increased relative to baseline during the recovery period for opsin-expressing animals, but not significantly so (Figure 8Ai, bout number, 13.9% increase, p = 0.58; Figure 8Bi, distance during locomotion, 3.7% increase, p = 0.161). However, we did not observe an increase in the vigour of locomotion, as measured by the mean bout speed and the mean distance travelled per bout (Figure 8Di, Ei). Also in agreement with our earlier findings, the stimulation-induced increases relative to baseline in bout number and total distance travelled during locomotion were not observed on OF Day 2. Rather, on OF Day 2 there was a separation in bout number and locomotion distance between groups that was statistically significant (bout number p = 0.001, locomotion distance p = 0.0317) (Figure 8Aii, Bii). These findings suggest that phasic SNCL DAN stimulation positively modulates locomotion initiation and perhaps total distance travelled. Neither stimulus-driven effect was replicated in either DLS implant group on either OF day (Figure 9A,B). There was a 21.3% decrease in bout length during the OF Day 1 stimulus period for the DLS opsin-expressing group (p = 0.01) that persisted into the recovery period (15.2% decrease relative to baseline, p = 0.0336) (Figure 9Ci). However, on the second exposure to the open field this trend was flipped between groups, with the opsin-expressing group showing no change in bout length and the opsin-negative group showing a significant decrease during the stimulus period (21.0% decrease, p = 0.0268) (Figure 9Cii). These occurrences of apparently stimulus-induced decreases in locomotion bout length (SNCL, Figure 8Ci,Cii; DLS, Figure 9Ci,Cii), and speed (SNCL, Figure 8Dii) that are replicated in opsin-positive and opsin-negative groups might be indicative of startle responses to the LED that warrant further investigation.

Given the similarity between the opsin-positive SNCL OF Day 2 stimulus-induced bout changes with the changes observed previously in rate of total horizontal movement, we next analyzed bout numbers within individual videos to assess whether these values were being affected by habituation, and whether video number correlated with an increased or decreased ability to

cause a change in locomotion (Figure 10). Interestingly, when averaged across the two days of OF exposure, we measured a 39% increase in bout number from baseline (p = 0.0684) on video 1 (min 0-9) in the opsin-expressing group, which was not observed in the opsin-negative group (Figure 10Ci), that mirrors the increase observed in the opsin-expressing group when looking at horizontal

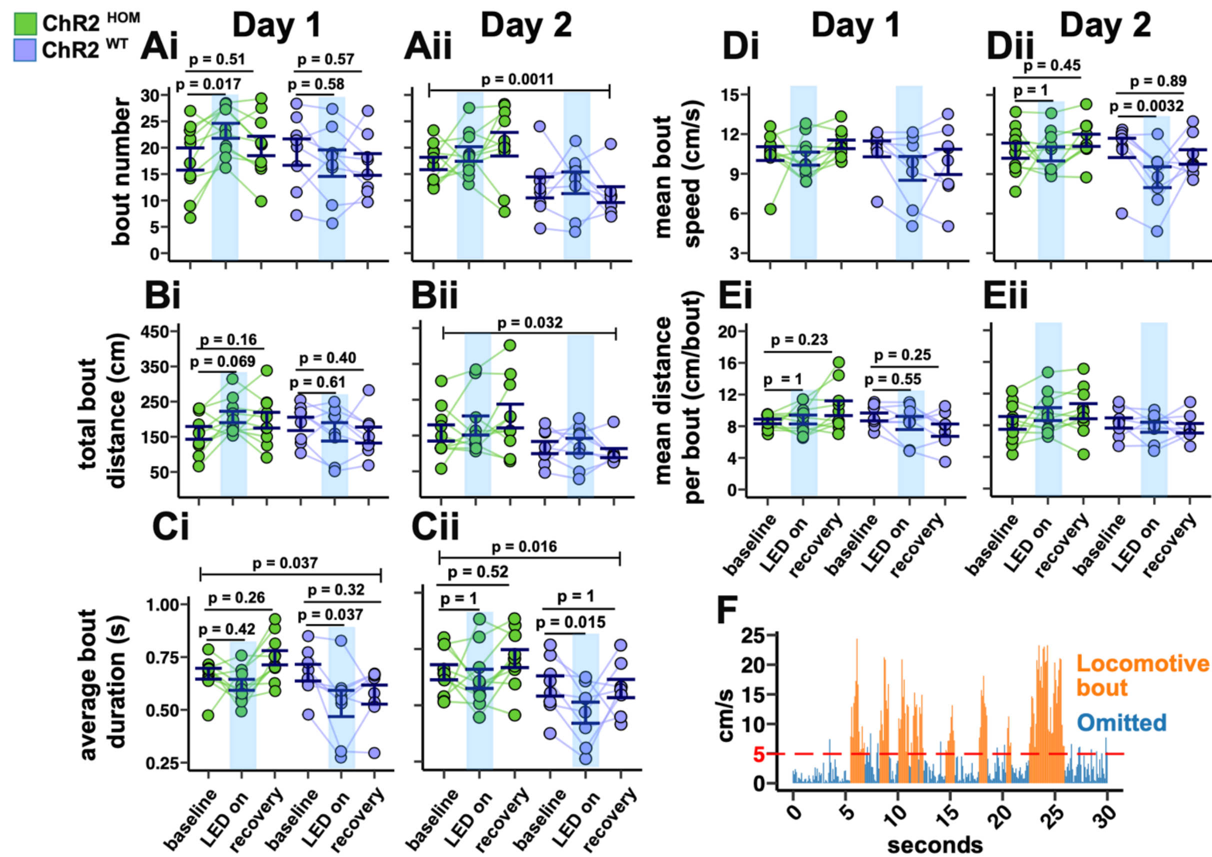


Figure 8. Stimulation of SNCL DANs differentially increases locomotion, but not locomotion vigour, depending on prior exposure to the open field. Values are averages of 4 consecutive videos. Locomotive bout data was collected from the last 90s of baseline, the 90s total stimulation period, and last 90s of recovery. Error-bars are the mean ± standard error of the mean. Opsin-positive animals: n = 10, 6 males, 4 females. Opsin-negative animals: n = 8, 4 males, 4 females. Number of locomotive bouts during measured during the first (**Ai**) and second (**Aii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA (**Ai and Aii**) followed by a post-hoc paired Student's T-test (**Ai**). Total distance travelled during locomotive bouts during the first (**Bi**) and second (**Bii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA (**Bi and Bii**) followed by a post-hoc paired Student's T-test (**Bi**). Average locomotive bout duration during the first (**Ci**) and second (**Cii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA followed by a post-hoc paired Student's T-test (**Ci and Cii**). Average locomotive bout speed during the first (**Di**) and second (**Dii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA (**Di and Dii**)

followed by a post-hoc paired Student's T-test (**Dii**). The average distance travelled per locomotive bout during the first (**Ei**) and second (**Eii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA (**Ei and Eii**) followed by a post-hoc paired Student's T-test (**Ei**). **F**. Example of a 30s LED-ON stimulus period with movement matching the criteria for locomotion ($\geq$ 5cm/s for $\geq$ 300ms) colored in orange and movement not matching the criteria colored in blue.

movement as a whole (Figure 6Ci, 30% increase, p = 0.065). Also similar to the total horizontal movement data, on OF Day 2 there was a robust separation between groups in overall bout number across time intervals that started at video 2 (min 10-19), which grew in statistical significance with each consecutive video (Figure 10B). Although the baseline bout numbers show the same trend as the total horizontal movement data, with a general decrease in the opsin-negative group that is not replicated in the opsin-expressing group, the difference between the video 4 baselines between groups did not reach statistical significance on either OF Day or when averaged across days (Figure 11 D, E, F). This could be indicative of the OF Day 2 separation in bout number being due to induced changes during the trial and recovery period. However, the statistically significant difference in OF Day 2 video 4 baseline horizontal activity levels between groups (p = 0.0291), although a large effect (Cohen's d = 1.03) was underpowered (1-β = 0.59) (Figure 7Bii) and the greater baseline bout number observed in the opsin-positive group relative to the opsin-negative group on OF Day 2 video 4 nearly reached statistical significance (Figure 11Bii, p = 0.0629). Also similar to the total horizontal movement findings, when averaged across days there was a significant increase in baseline bout numbers between the first and second videos (min 0-3 and 10-13) for the opsin-expressing group (Figure 7Ci, horizontal movement, 39.7% increase, p = 0.00585; Figure 11Ci, bout number, 53.0% increase, p = 0.0411). Together I think this data suggests that phasic stimulation of SNCL DANs increases initiation of locomotion in a novelty-dependant manner, with a more direct promotion of locomotion in a novel environment and inhibition of normal habituation to the environment as novelty decreases in the second exposure.

3.2 Stimulation of lateral SNC DANs causes an increase in non-locomotive exploratory behaviour that is novelty-dependant.

An element of movement not captured by our Image-J-based analysis of horizontal movement and locomotion is exploratory behaviour that involves vertical movements. While exploring their

environment mice will often rear on their hind legs to gain more information about a novel setting, and in fact rearing is itself considered a reliable marker of environmental novelty (Lever *et al.,* 2006). In addition, how an animal rears, with its paws on the wall (supported) or not (unsupported), is differentially affected by the stress levels of an animal, with unsupported rears being more stress-sensitive (Sturman *et al.*, 2018). Furthermore, when not exploring their environment mice will

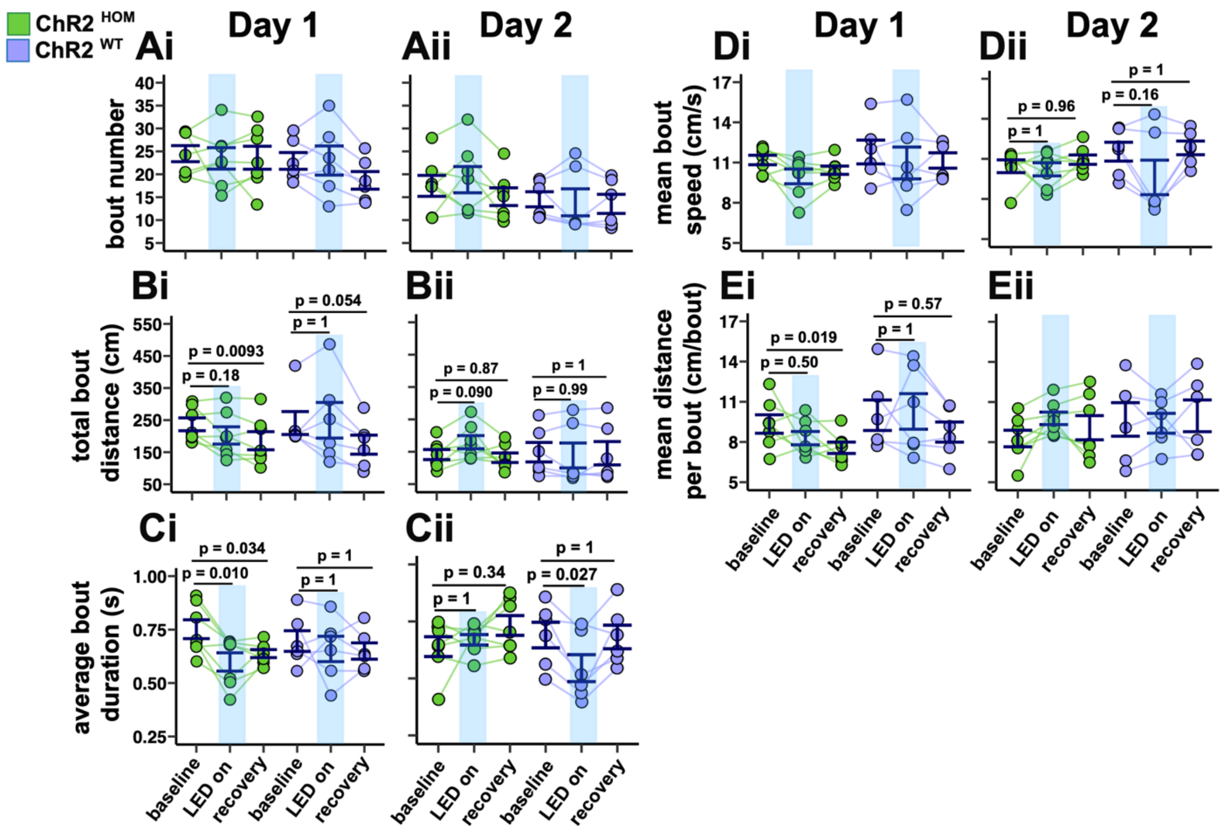


Figure 9. Stimulation of DAN terminals in the DLS does not positively modulate locomotion. Values are averages of 4 consecutive videos. Locomotive bout data was collected from the last 90s of baseline, the 90s total stimulation period, and last 90s of recovery. Error-bars are the mean ± standard error of the mean. Opsin-positive animals: n = 7, 3 males, 4 females. Opsin-negative animals: n = 6, 2 males, 4 females. Number of locomotive bouts during measured during the first (**Ai**) and second (**Aii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA. Total distance travelled during locomotive bouts during the first (**Bi**) and second (**Bii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA followed by a post-hoc paired Student's T-test. Average locomotive bout duration during the first (**Ci**) and second (**Cii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA followed by a post-hoc paired Student's T-test. Average locomotive bout speed during the first (**Di**) and second (**Dii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA (**Di and Dii**) followed by a post-hoc paired Student's T-test (**Dii**). The average distance travelled per locomotive bout during the first (**Ei**) and second (**Eii**) exposure to the OF.

Comparisons were made with a two-way mixed repeated ANOVA (**Ei and Eii**) followed by a post-hoc paired Student's T-test (**Ei**).

often groom as an important, normal behaviour and also notably to alleviate stress (Smolinsky *et al.,* 2009; Berry *et al*., 2012). To account for these non-locomotive behaviours, the researcher was blinded to the identity and genotype of the animals and manually tabulated the occurrence of each behaviour (supported rear, unsupported rear, grooming). We also measured escape behaviours such as attempting to climb and jump out of the OF arena, but as these were very rare, they were accounted for only in summated measures of total non-locomotive activity (Figures 11,12A).

In agreement with the total horizontal movement and locomotion data, phasic stimulation of SNCL DANs resulted in a significant increase in non-locomotive activity on the first exposure to the open field. We observed a robust 32.6% ($p = 0.0138$) increase from baseline in total non-locomotive events in opsin-expressing SNCL implant animals that was not observed in opsin-negative SNCL implant animals (6% increase, $p = 0.724$) (Figure 12Ai) or in either DLS implant group (Figure 13Ai). This stimulus-induced increase in non-locomotive activity in the opsin-expressing SNCL group can be attributed in large part to supported and unsupported rearing, as both increased significantly during the stimulus period (40.2% increase, $p = 0.00278$) and remained elevated during the recovery period (27.3% increase from baseline, $p = 0.0446$) (Figure 11Di). The opsin-negative SNCL implant group did not exhibit this same increase during the stimulus (6.1% decrease, $p = 1$) or recovery period (5.2% decrease, $p = 1$) (Figure 12Di). Interestingly, grooming activity was not affected by stimulus (Figure 12Ei). On OF Day 2, unlike the horizontal movement and locomotion data, there was no statistically significant separation in any non-locomotive activity counts between SNCL implant groups, although there may be a trend of some separation. On OF Day 2, the summed non-locomotive activity was almost significantly higher relative to baseline for the opsin-expressing group during the stimulus period (21.1% increase, $p = 0.0846$) (Figure 12Aii), however, it was not accompanied by any stimulus-induced increase in rearing. There was no change in non-locomotive activity during stimulus for either DLS implant group on OF Day 1 or 2 (Figure 13). These results suggest that phasic activation of SNCL DANs powerfully promotes exploratory behaviours like rearing and that this affect is highly dependent on the novelty of the animal's surroundings.

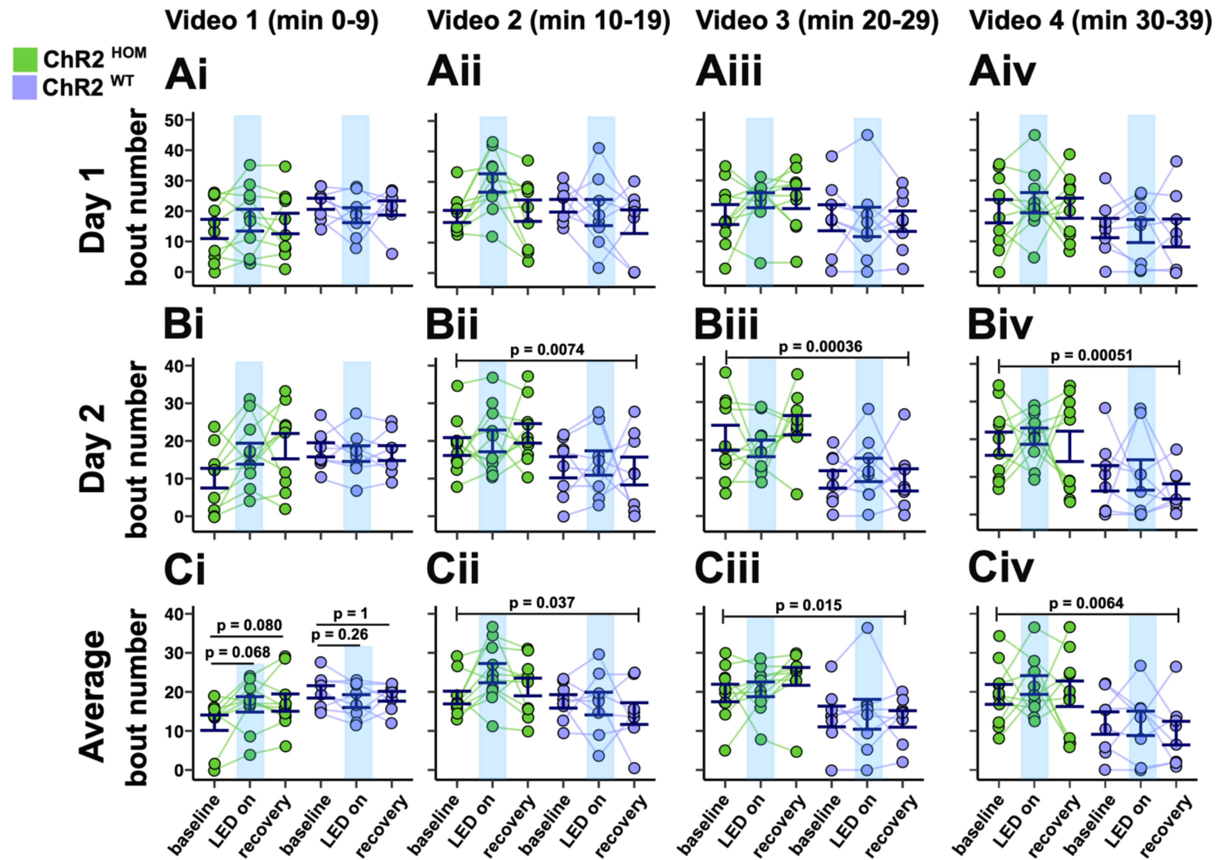


Figure 10. Stimulation of SNCL DANs differentially inhibits the habituation of locomotive initiation depending on prior exposure to the open field. Locomotive bout numbers were obtained from the last 90s of baseline, the 90s total stimulation period, and last 90s of recovery. Error-bars are the mean ± standard error of the mean. Opsin-positive animals: n = 10, 6 males, 4 females. Opsin-negative animals: n = 8, 4 males, 4 females. Locomotive bout number during the first day of OF exposure during the first (**Ai**), second (**Aii**), third (**Aiii**), and fourth (**Aiv**) recording session. Locomotive bout number during the second day of OF exposure during the first (**Bi**), second (**Bii**), third (**Biii**), and fourth (**Biv**) recording session. Locomotive bout number averaged across the two days of OF exposure during the first (**Ci**), second (**Cii**), third (**Ciii**), and fourth (**Civ**) recording session. Comparisons were made with a two-way mixed repeated ANOVA followed by a post-hoc Student's T-test with Bonferroni correction (**Ci**).

Chapter 4. Discussion

There is a large precedent in the scientific body that phasic dopaminergic signalling along the nigrostriatal pathway plays an important role in the initiation of voluntary actions (Jin and Costa, 2010; Kravitz *et al*., 2010). However, how the SNC contributes to this function remains unclear, likely contributed, in part, by the heterogenous nature of the SNC and its downstream projections in the DS. In this study we attempt to draw a link between work demonstrating that the lateral nigrostriatal pathway is potentially functionally distinct (Schiemann *et al*., 2012; Lerner and Deisseroth, 2015; Estakhr *et al*., 2017) and optogenetic behaviour experiments investigating phasic dopamine release in the DS (Howe and Dombeck, 2016) and SNC (Da Silva *et al.,* 2018) and its relationship with modulating voluntary movement. Additionally, in what we believe is a first in optogenetic stimulation of nigrostriatal circuits, we have incorporated the knowledge that phasic SNC DAN activity encodes contextual information about the environment, and can modulate responses to changing context. We found that phasic stimulation of DAN somata in the lateral SNC increased exploratory behaviour, specifically the rate of horizontal, or translational, activity, locomotive bout number, and supported and unsupported rearing, on the first day of exposure to the OF. On the second day of OF exposure we observed a lack of habituation in the rate of horizontal movement and in locomotive bout number, but not in exploratory behaviours with a vertical component, *i.e*., rearing. Our findings suggests that the motivational context of novelty is an important variable intersecting with phasic dopamine signalling and must be considered when investigating how dopamine modulates voluntary, naturalistic behaviours.

4.1 Phasic stimulation of SNCL DANs increases voluntary exploratory activity in a novel environment.

Using implanted optic fibres delivering blue LED light, we stimulated a transgenic mouse model conditionally expressing cre-recombinase-driven ChR2 in dopaminergic neurons. While stimulating mice as they explored an open field, we observed an increase in exploratory activity (rate of horizontal movement, locomotive bout number, rearing). Not affected were behaviours unrelated to gaining information about the environment, *i.e*., grooming. These results are consistent with previous work suggesting that phasic dopamine stimulation promotes and even drives voluntary locomotion (Howe and Dombeck, 2016; Da Silva *et al.,* 2018). However, our

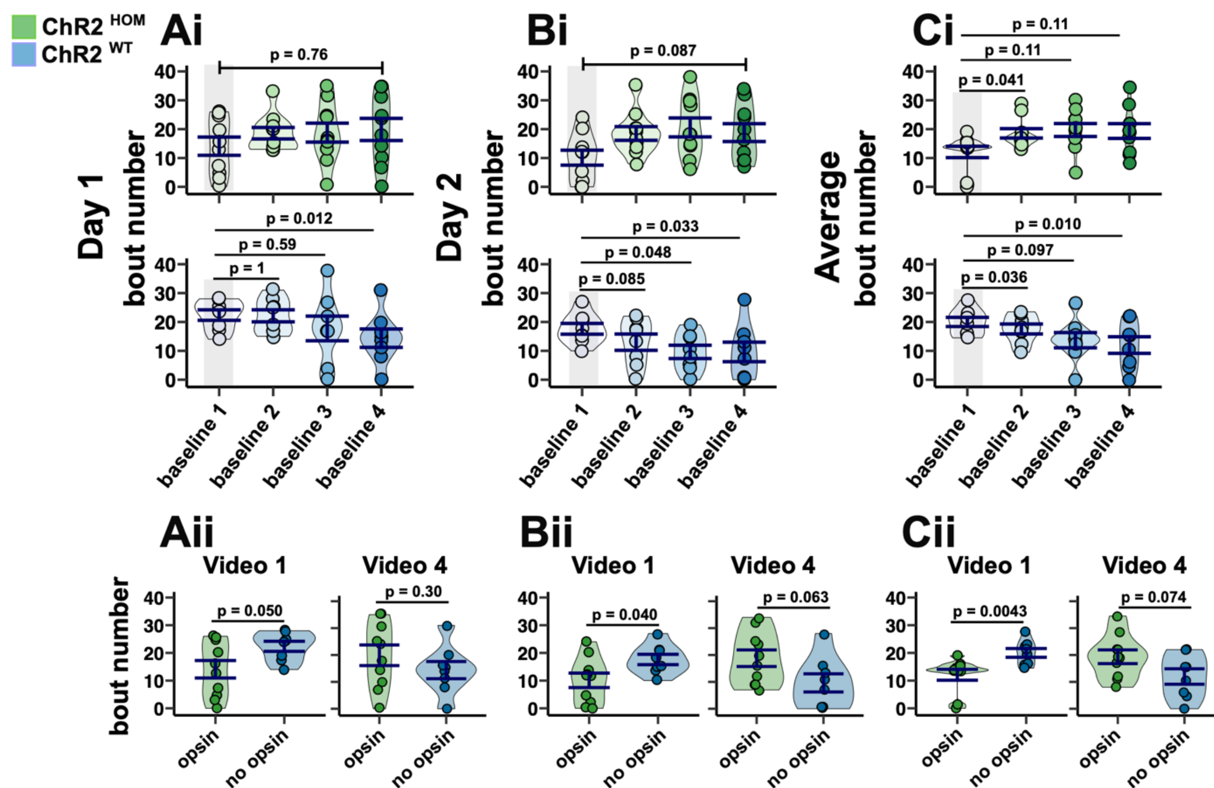


Figure 11. Lack of Habituation in SNCL-stimulated animals can be attributed to a lack of decrease in baseline locomotion throughout open field exposure. Locomotive bout numbers were obtained from the last 90s of baseline, the 90s total stimulation period, and last 90s of recovery. Error-bars are the mean ± standard error of the mean. Opsin-positive animals: n = 10, 6 males, 4 females. Opsin-negative animals: n = 8, 4 males, 4 females. Baseline activity occurs over minutes 0-3 (baseline 1), 10-13 (baseline 2), 20-23 (baseline 3), and 30-33 (baseline 4). **Ai.** Baseline bout numbers across 4 consecutive recording sessions during the first exposure to the OF. Comparisons were made with a Freidman's test (opsin-positive) and a one-way repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction (opsin-negative). **Bi**. Baseline bout numbers across 4 consecutive recording sessions during the second exposure to the OF. Comparisons were made with a one-way repeated ANOVA (opsin-positive and negative) with a post-hoc paired Student's T-test with Bonferroni correction (opsin negative). **Ci.** Baseline bout numbers across 4 consecutive recording sessions averaged across the 2 days of OF exposure. Comparisons were made with a one-way repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction (opsin-positive and negative). Comparison of the first and fourth baseline bout numbers between groups during the first exposure to the OF (**Aii**), the second exposure to the OF (**Bii**) and when averaged across the 2 days of exposure (**Cii**). Comparisons between groups were done with an unpaired Student's T-test (**Aii, Bii**) and an unpaired Wilcoxon Rank Sum Test (**Cii**).

findings suggests that there might be a specific exploring, or novelty-seeking, functionality that is being promoted by phasic activity. This is supported by the work of Schiemann *et al*., (2012) where they observed K-ATP-mediated burst firing of SNC DANs only mediated higher exploration, measured as changes in locomotion and rearing, than knock-out animals on the first day of OF exposure. Interestingly, they reported that silencing of K-ATP-mediated burst firing in the SNCL had no effect on exploration, regardless of the day tested. Coupled with our findings, this seems suggestive of a possibly different mechanism mediating burst firing-driven exploration in the SNCL. Our results are also consistent with other studies linking increased dopaminergic signalling, specifically increased dopamine tone in the striatum, with increased exploratory behaviours (Pogorelov *et al*., 2005; Costa *et al*., 2014; Leach *et al*., 2022).

Our finding that we could only induce an increase in exploration for a limited period of time following exposure to a novel open field is supported by other studies (Giménez-Llort *et al*., 1997; Schiemann *et al*., 2012). This suggests that there is an intersection of novelty, or motivating contextual cues, with phasic firing that drives behaviour change. As we were unable to cause an increase in exploratory activity that was time locked to the stimulus period on the second day of exposure, this is suggestive that phasic stimulation is not sufficient on its own to overcome a lack of motivational context.

4.2 Phasic stimulation of SNCL DANs in low-novelty environment inhibits habituation.

It is interesting that on both days of OF exposure we observed a lack of locomotive habituation in the stimulated group (separation in overall activity by video 4, min 30-39), but the difference in baseline activity (by min 30) only became significantly different from the control group on the second day of OF exposure. The lack of habituation was indicated by a lack of decrease in baseline activity (rate of horizontal movement and bout number) across consecutive recording sessions (videos), and even an increase between the baselines of the first and second recording session. Interestingly, we did not observe this lack of habituation in the other main exploratory behaviour: rearing. The observed lack of habituation in the stimulated group is consistent with other work wherein prevention of dopamine uptake in the striatum reduced intrasession habituation (Giros *et al*., 1996; Gainetdinov *et al*., 1999). However, these accounts coincide with increased motor activity, and knock-out of dopamine receptors was observed to have the same affect (Wong *et al*.,

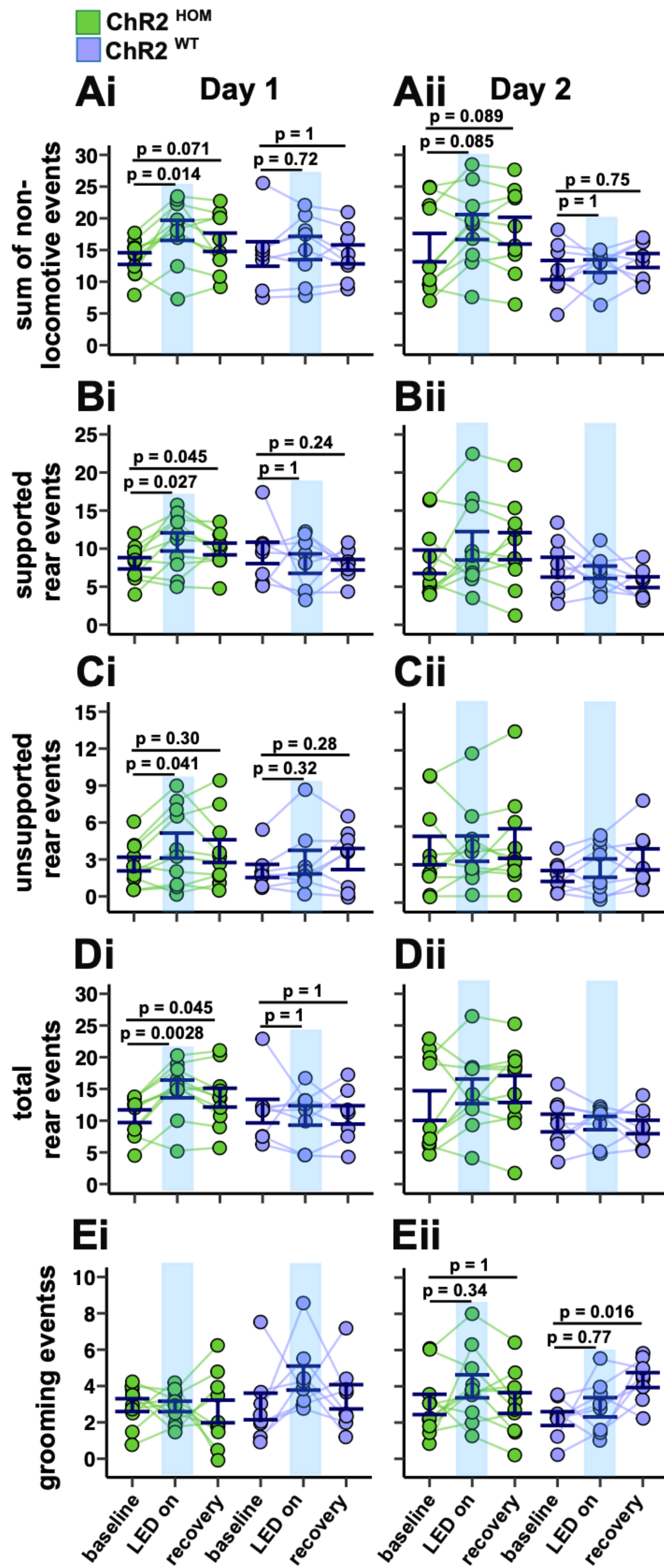


Figure 12. Stimulation of SNCL DANs increases non-locomotive activity in a novelty-dependant manner. Data points represent values for individual animals averaged across 4 consecutive videos. Error bars represent the mean ± the standard error of the mean. Opsin-expressing animals: n = 10, 6 males, 4 females; opsin-negative animals: n = 8, 4 males, 4 females. Non-locomotive behaviours were obtained from full 3 min baseline, trial, and recovery periods. Total number of non-locomotive behaviours (supported rears, unsupported rears, grooming, escape behaviours) during the first (**Ai**) and second (**Aii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction. Number of supported rearing events during the first (**Bi**) and second (**Bii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction (**Bii**). Number of unsupported rearing events during the first (**Ci**) and second (**Cii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction (**Ci**). Total number of rearing events, supported and unsupported, during the first (**Di**) and second (**Dii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction (**Di**). Number of grooming events during the first (**Ei**) and second (**Eii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction (**Eii**).

2003). It is plausible that the movement-promoting signal is acting over a time frame longer than the stimulus period so that the movement-promoting ChR2 stimulation of DANs counteracts the underlying habituation. Dopamine signalling has been posited to encode different things depending on the time-frame over which it is acting, and indeed changes in dopaminergic concentrations accompanying movement as measured by voltammetry and micro dialysis have been measured over minutes to tens-of-minutes (Schultz, 2007: Markowitz *et al*., 2023). In addition, long-term potentiation of glutamatergic synapses on MSNs can last 10's of min (Shen *et al*., 2008). This idea would be in line with our findings that stimulation results in a carry-over of increased locomotion and rearing into the recovery period on the first exposure to the OF. This is consistent with the findings by Markowitz *et al*., (2023), who showed that behaviours that were coincident with dopamine transients in the striatum were reinforced in subsequent minutes of behaviour. When we are phasically stimulating the animals, we are stimulating them while they are actively moving and exploring, especially on the first exposure, or re-exposure, to the OF. The stimulation, when it coincides with exploratory behaviour, perhaps promotes and reinforces these behaviours in subsequent recovery and baseline periods, as the animals are internally motivated to replicate behaviours that coincided with the stimulated dopamine transients.

There is also a possibility that the lack of habituation observed is due to a dysregulation of normal memory formation, or learning, as the DS (De Leonibus *et al*., 2005; Doeller *et al*., 2008; Méndez-Couz *et al.*, 2015) and specifically lateral nigrostriatal dopamine has been shown to have a critical role in spatial and object memory. A dopamine-deficient mouse model in which dopamine signalling was restored in the DLS-projecting DANs was shown to have restored motivation to work for food, restored cue-dependant learning, and partially-restored object and visuospatial learning (Darvas and Palmiter, 2009).

4.3 Phasic stimulation of SNCL DANs increases locomotion by increasing the number of initiations of locomotion, rather than increasing locomotion vigour.

An interesting result from this experiment was the observation that phasic stimulation, in the novel condition of first-exposure to the OF, increased locomotion by specifically increasing the number of times the stimulated animals started the action sequence of walking (bout number), as opposed

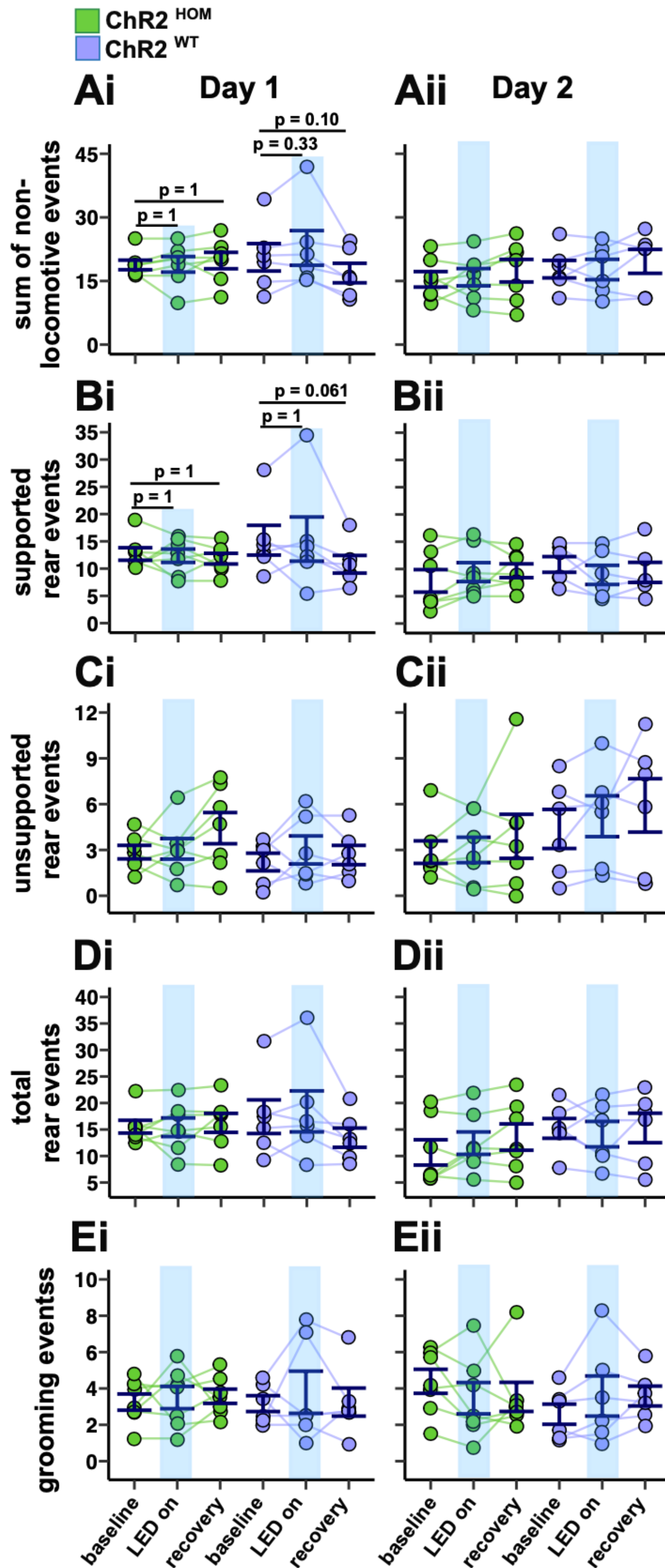


Figure 13. Stimulation of DAN axon terminals in the DLS has no effect on non-locomotive events. Data points represent values for individual animals averaged across 4 consecutive videos. Error bars represent the mean ± the standard error of the mean. Opsin-expressing animals: n = 7, 3 males, 4 females; opsin-negative animals: n = 6, 2 males, 4 females. Non-locomotive behaviours were obtained from full 3 min baseline, trial, and recovery periods. Total number of non-locomotive behaviours (supported rears, unsupported rears, grooming, escape behaviours) during the first (**Ai**) and second (**Aii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction (**Ai**). Number of supported rearing events during the first (**Bi**) and second (**Bii**) exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA with a post-hoc paired Student's T-test with Bonferroni correction (**Bi**). Number of unsupported rearing events during the first (**Ci**) and second (**Cii**) exposure to the OF. Total number of rearing events, supported and unsupported, during the first (**Di**) and second (**Dii**) exposure to the OF. Number of grooming events during the first (**Ei**) and second (**Eii)** exposure to the OF. Comparisons were made with a two-way mixed repeated ANOVA (**Ci-Eii**).

to increasing the vigour of locomotion. This is interesting as it conflicts with literature that indicates that SNCL DANs encode aspects of movement vigour (Panigrahi *et al*., 2015; Howe and Dombeck, 2016; da Silva *et al*., 2018), but adds to evidence that phasic dopaminergic signalling in the SNC is important for initiating voluntary movements (Jin and Costa, 2010). It is possible that we would observe a positive change in short-term movement vigour if we measured over the same small time-scales that other researchers have (Howe and Dombeck, 2016; Da Silva *et al*., 2018; Markowitz *et al.,* 2023).

A perplexing aspect of this finding, that phasic activation of SNCL DANs increases the number of locomotive bouts, is that we were unable to observe similar findings in animals with optic fibre implants placed over the DLS. This was unexpected given our knowledge that the DLS is the main downstream target of the SNCL, and that the DLS is where the initiation and termination of well-learned action sequences, such as walking, are thought to be encoded. There are possible explanations for this discrepancy. Although we verified implant placement to be in the DLS, and targeted similar stereotaxic coordinates and depths as other studies optogenetically stimulating SNCL DAN axons in the DLS (Lerner *et al*., 2015), it is possible that the location of where we stimulated in the SNL does not project in the location that we stimulated in the DLS, but elsewhere in the DLS. To ascertain with certainty the optimal localization in the DLS for optic fiber implants, we would need to stereotaxically inject anterograde tracers in the SNL and correspondingly retrograde tracers in the DLS. Another explanation could be that there is evidence that nigrostriatal axonal arborizations can be quite diffuse (Prensa and Parent, 2001), while the cell bodies in the SNCL are quite compact. Therefore, in theory stimulation in the SNCL should excite more neurons than stimulating directly the more diffuse projection terminals in the DLS.

4.4 The Role of the lateral SNC in Modulating Voluntary Behaviour: A Potential Model

In the many decades that researchers have studied the nigrostriatal pathway and its relation to voluntary movement, there are a few ideas that seem resilient. At the center appears to be the concept that the lateral nigrostriatal pathway is an integration center that incorporates temporally-relevant cues, such as salient stimuli from the environment (Horvitz, 2000), the motivational state

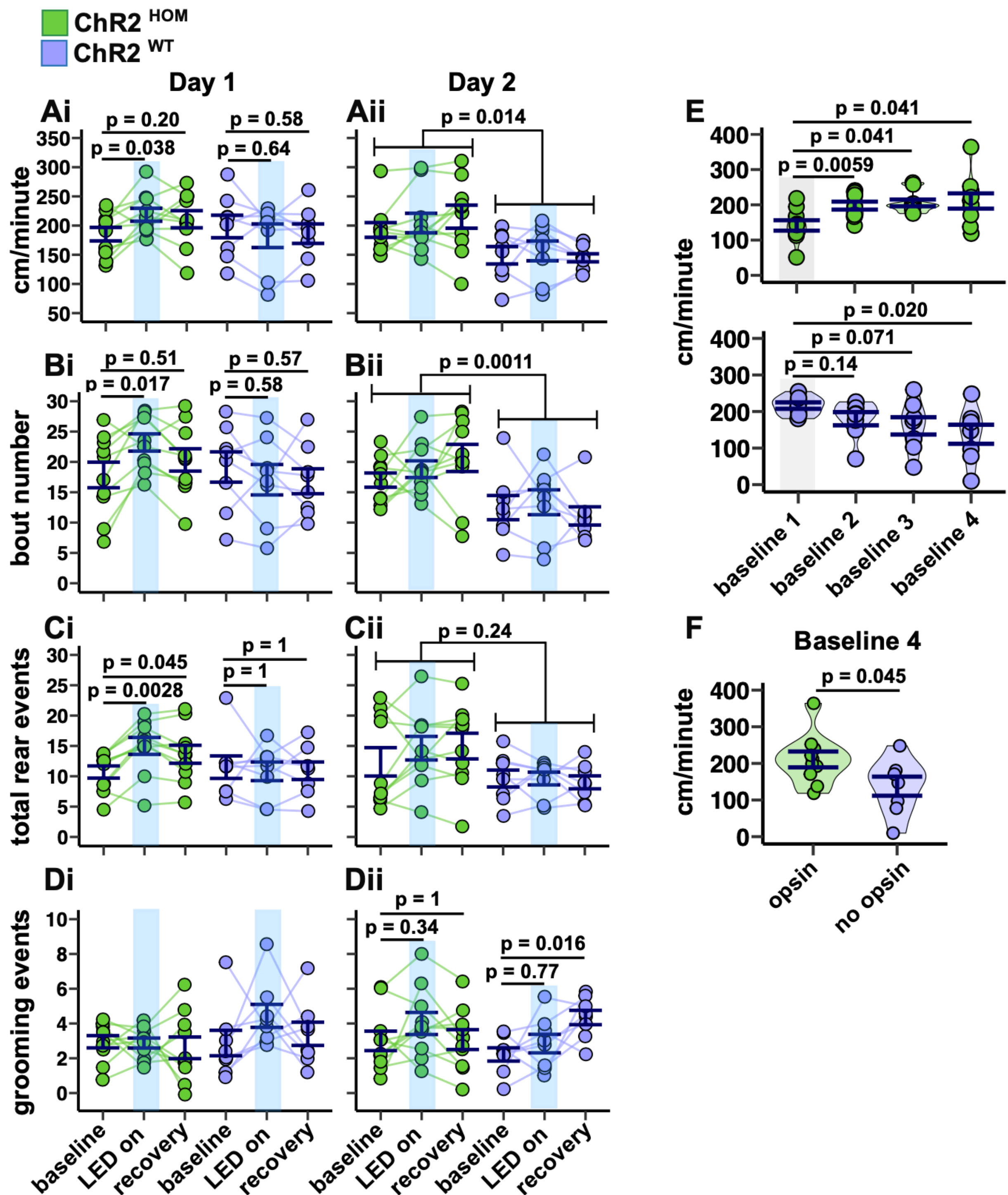


Figure 14. Phasic stimulation of SNCL DANs promotes exploratory behaviour specifically in a novel environment and interferes with habituation. Data points represent values for individual animals averaged across 4 consecutive videos. Error bars represent the mean ± the standard error of the mean. Green: opsin-positive (n = 10); blue: opsin-negative (n = 8). **Ai.** Normalized rate of total horizontal activity during the first exposure to the OF. Figure panel taken

from Figure 5Ai. **Aii**. Values from second exposure to the OF. Figure panel from Figure 5Aii. **Bi.** Number of locomotive bouts initiated during the first exposure to the OF. Figure panel from Figure 8Ai. **Bii**. Values from second exposure to the OF. Figure panel from Figure 8Aii **Ci.** Supported and unsupported rear events during the first exposure to the OF. Figure panel from Figure 12Di. **Cii.** Values from second exposure to OF. Figure panel from Figure 12Dii. **Di.** Grooming events during the first exposure to the OF. Figure panel from Figure 12Ei. **Dii.** Values from second exposure to the OF. Figure panel from Figure 12Eii. **E.** Opsin-positive (green) and opsin-negative (blue). Normalized rates of total horizontal activity during baseline averaged across 2 consecutive days of O Fexposure. Figure panel from Figure 7Ci. **F.** Comparison of normalized baseline rates of activity between opsin-positive and opsin-negative animals during the baseline of the fourth video of the recording session (min 30-33) averaged across 2 consecutive days of OF exposure. Figure panel from Figure 7Cii.

of the animal (Matsumoto and Hikosaka, 2009), and the animal's prior experiences (Schult *et al*., 1997), and with this information adjusts the permissive "gain" of striatal excitability (Galarraga *et al*., 1997; Cepeda *et al*., 1998) that leads to downstream movement. The various intersecting elements of information may be separated by the time over which dopamine transients from the SNCL are encoded. At the most temporally immediate, phasic DAN activity in the SNCL provides a context-dependant, movement-motivating cue. For example, there is a novel stimuli that has the potential to be rewarding (Redgrave *et al*., 2008; Krebs *et al*., 2011; Menegas *et al*., 2017). This motivating signal is intrinsically rewarding and encourages the animal to engage with that motivation. If the cue is novelty, the animal will be motivated to explore (Kakade and Dayan, 2002; Hazy *et al*., 2010). If the cue is an extrinsic reward, the animal will be motivated to approach (Schultz, 1997, 2000). Over a slightly longer timescale, behaviours that coincide with these intrinsically "rewarding" dopaminergic transients are reinforced over subsequent minutes (Reynolds *et al*., 2001; Markowitz *et al*., 2023). On a longer timescale, if the behaviour is repeatedly successful, or rewarding, over multiple occasions then the behaviour is "cemented" in the DLS, the home of "start-stop" signals, as a habit that is no longer dependant on outcome (Jin and Costa, 2010; Smith and Graybiel, 2013a). This successive reinforcement of behaviours that coincide with SNCL-DLS dopamine transients, regardless of the context that causes the transient, I think may explain the ability of phasic SNCL DAN signalling to promote behaviours that are novelty-dependant over shorter timescales, but with repetition, are resistant to habituation over longer timescales.

4.5 Future Directions

The findings from this study open up a number of intriguing avenues of inquiry. Phasic stimulation of SNCL DANs seems to selectively promote exploratory activity for a limited time after exposure to a novel environment. A novel object test, where stimulated animals have the option to continue moving in the OF or investigate a novel object introduced to the OF, could help us to be more certain that exploratory activity is being selected for with phasic stimulation.

We observed that phasic stimulation only increased exploratory activity during the stimulus period on the first day of OF exposure and not on the second day. It is possible that there was no response to stimulation on the second day because the environment was no longer novel, but there is also the possibility that some long-term plasticity change occurred following stimulation during the first OF exposure. To clarify the reason behind this finding, it would be prudent to have a cohort of animals exposed to the OF for two consecutive days, as was done in this study, but only stimulated on the second exposure day. If the results are consistent with the OF Day 2 results from this study, it suggests that a lack of novelty and internal motivation is the cause for the lack of movement promotion, as opposed to neuronal habituation to the LED stimulus. In addition, a sub-group of animals on the second day could receive a novel object on OF Day 2. In theory, if the motivating element of novelty is a requirement for phasic stimulation-induced movement, this should "rescue" the behavioral phenotype observed in OF Day 1 of the present study.

The time-frame over which dopamine signalling encodes different information is an active field of discussion and research. Our findings suggest that there are perhaps multi-min spanning changes occurring, as evidenced by the bleed-over of increased activity following stimulation on the first day of OF exposure and the lack of habituation on the second day of OF exposure. Although this study was focused on exploratory behaviours that require more than secs or sub-secs to be measured, it would also be interesting to look at sec/sub-sec movement changes in response to stimulus over periods of decreasing environment novelty to see if there is a more "direct" promotion of movement as seen in some studies (Howe and Dombeck, 2016).

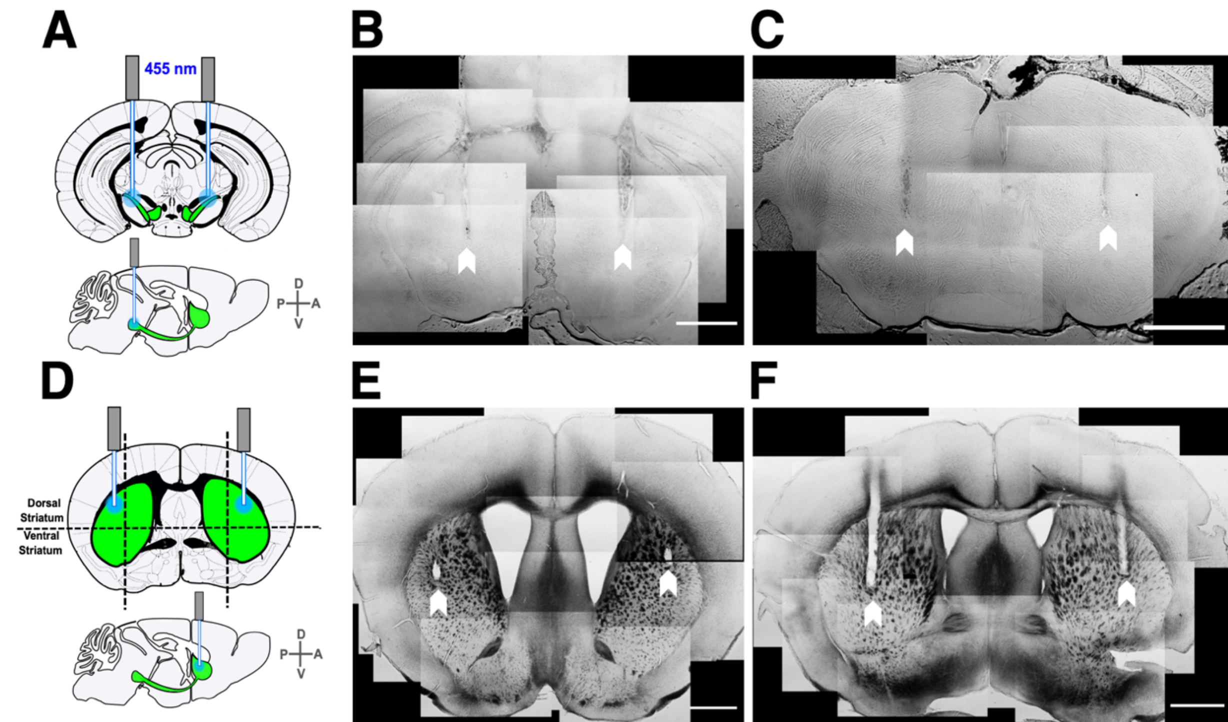
The focus of this study was on the lateral nigrostriatal pathway. However, it would also be interesting to repeat these experiments in the medial nigrostriatal pathway, as the VTA/medial nigrostriatal pathway has been shown to have a role in encoding contralateral action selection and

movement (Parker *et al*., 2016; Lee *et al.*, 2019). As the lateral and medial nigrostriatal pathways appear to be functionally segregated (Lerner *et al.,* 2015) it would be interesting to investigate whether they have different roles in modulating voluntary movement. In order to target DANs of the medial SNC (SNCM) specifically, and not target the VTA as well, it would be necessary to deliver the opsin via injection of a retrograde virus in the DMS.

Finally, it would be interesting to perform anterograde and retrograde viral tracing experiments at the stereotaxic coordinates used in this experiment, in order to anatomically confirm exactly where in the DLS is the best location for placement of optic fibres to target SNCL axon terminals.

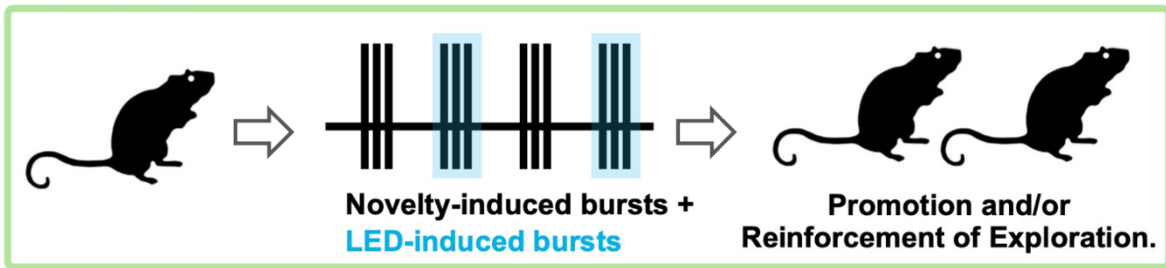
Chapter 5. Concluding Remarks

In this study we demonstrated that phasic photoactivation of dopaminergic somata in the lateral SNC promotes exploratory behaviour in a novelty-dependant manner. This work builds on a body of literature demonstrating that phasic activity in the dopaminergic nigrostriatal pathway promotes voluntary movement, specifically the initiation of movements. This work is novel in that it expands the field of phasic optogenetic stimulation of nigrostriatal DANs to include the measuring of naturalistic behaviours like exploring. Furthermore, we have incorporated the knowledge that DAN signalling intersects with novelty encoding and the shaping of behaviours in response to novelty. Our results show that context is intimately involved in the type of behaviours that are driven by phasic activation of midbrain dopamine neurons, and that the time courses over which phasic dopamine modulates behaviour needs to be investigated in greater detail.

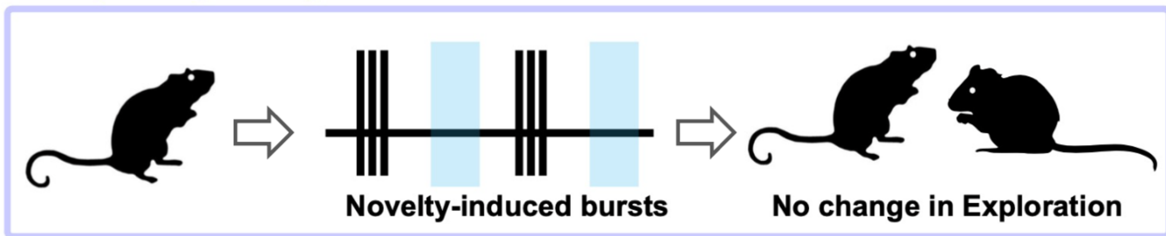


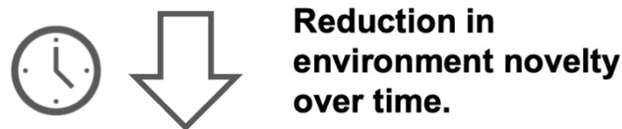
Supplementary Figure 1. Examples of optic fibre tracks. Schematic of SNCL (**A**) and DLS (**D**) target brain region. Green indicates dopaminergic neurons expressing ChR2-EYFP. 4x bright-field image of DATcre::ChR2-EYFP (**B**) and DATcre (**C**) animal with fibre optic tracks over the SNCL. 4x bright-field image of DATcre::ChR2-EYFP (**E**) and DATcre (**F**) animal with fibre optic tracks in DLS. White arrows indicate track terminus. Scale bars 1mm.

Opsin-expressing

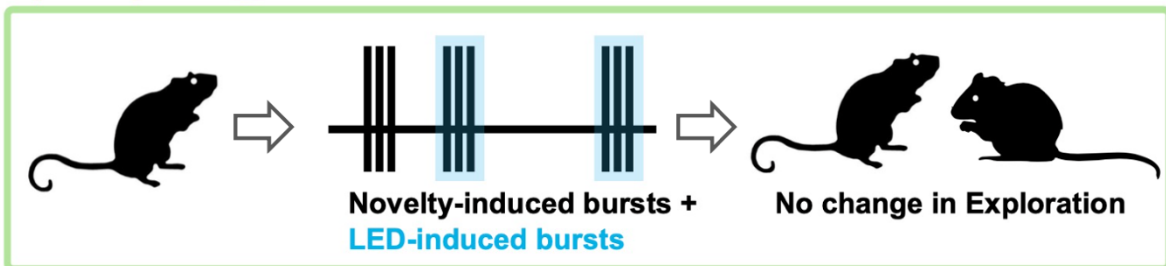


Non Opsin-expressing

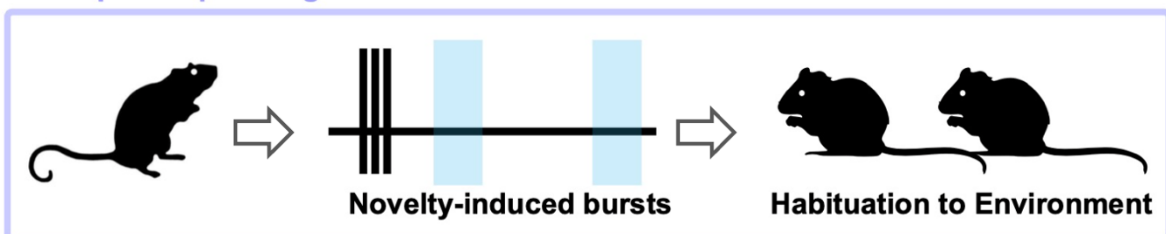




Opsin-expressing



Non Opsin-expressing



Supplementary Figure 2. Proposed model of the effect of phasic stimulation over time. Blue shading represents LED stimulation. Phasic stimulation of SNCL DANs coinciding with novelty-driven endogenous bursts of DAN activity promotes novelty-prompted exploratory behaviour. Over time as novelty decreases, phasic activity no longer increases exploratory behaviour, but rather interferes with habituation.

Table S1. Statistical Analyses related to Figures 5-13.

Figure	Sample Size	Panel	Statistical Test	Values
Figure 5	Opsin +ve: n = 10 Opsin -ve: n = 8	Ai	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,16) = 0.69$, $p = 0.42$ LED: $F(2,32) = 0.49$, $p = 0.62$ Genotype x LED: $F(2,32) = 3.94$, $p = 0.03$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -2.86$, $p = 0.0189 \times 2 = 0.038$ Opsin +ve baseline-recovery: $t(9) = -1.84$, $p = 0.0989 \times 2 = 0.20$ Opsin -ve baseline-trial: $t(7) = 1.075$, $p = 0.318 \times 2 = 0.64$ Opsin -ve baseline-recovery: $t(7) = 1.14$, $p = 0.290 \times 2 = 0.58$
		Aii	Two-way mixed repeated ANOVA.	Genotype: $F(1, 16) = 7.702$, $p = 0.014$ LED: $F(2, 32) = 0.67$, $p = 0.52$ Genotype x LED: $F(2, 32) = 1.181$, $p = 0.32$
		Aiii	Day 1: Parametric one-way repeated ANOVA for opsin +ve group, non-parametric Friedman's test for opsin -ve group. Day 2: Parametric one-way repeated ANOVA for opsin +ve group, non-parametric Friedman's test for opsin -ve group.	Day 1: LED: $F(3, 27) = 2.075$, p = 0.13 $\chi^2(3) = 7.05$, $p = 0.07$ Day 2: LED: $F(3,27) = 0.75$, $p = 0.53$ $\chi^2(3) = 0.9$, $p = 0.83$
	Opsin +ve: n = 7 Opsin -ve: n = 6	Bi	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,11) = 1.18\text{e-}03$, $p = 0.97$ LED: $F(2,22) = 7.011$, $p = 4.41\text{e-}03$ Genotype:LED: $F(2,22) = 4.164$, $p = 0.029$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(6) = 2.29$, $p = 0.0624 \times 2 = 0.13$ Opsin +ve baseline-recovery: $t(6) = 2.52$, $p = 0.0456 \times 2 = 0.091$ Opsin -ve baseline-trial: $t(5) = 0.23$, $p = 0.826 \times 2 = 1.65$ Opsin -ve baseline-recovery: $t(5) = 3.94$, $p = 0.0110 \times 2 = 0.022$

		Bii	Two-way mixed repeated ANOVA.	Genotype: $F(1,11) = 0.77$, $p = 0.40$ LED: $F(1,11) = 1.94$, $p = 0.17$ Genotype x LED: $F(2,22) = 1.58$, $p = 0.23$
		Biii	Day 1: Parametric one-way repeated ANOVA for opsin +ve and opsin -ve groups. Day2: Parametric one-way repeated ANOVA for opsin +ve group, non-parametric Friedman's test for opsin -ve group.	Day 1: Opsin +ve: LED: $F(3,20) = 0.92$, $p = 0.45$ Opsin -ve: LED: $F(3,16) = 0.071$, $p = 0.97$ Day2: LED: $F(3,20) = 0.018$, p= 0.10 $\chi^2(3) = 1.4$, $p = 0.71$
	Opsin +ve: n = 8 Opsin -ve: n = 8	Ci	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,14) = 0.018$, $p = 0.90$ LED: $F(2,28) = 15.22$, $p = 3.37e\text{-}05$ Genotype x LED: $F(2,28) = 0.64$, $p = 0.54$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(7) = 2.83$, $p = 0.0254 \times 2 = 0.051$ Opsin +ve baseline-recovery: $t(7) = 3.20$, $p = 0.0151 \times 2 = 0.03$ Opsin -ve baseline-trial: $t(7) = 2.80$, $p = 0.0265 \times 2 = 0.053$ Opsin -ve baseline-recovery: $t(7) = 5.14$, $p = 0.00134 \times 2 = 0.0027$
		Cii	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,14) = 3.76$, $p = 0.073$ LED: $F(2,28) = 5.77$, $p = 7.96e\text{-}03$ Genotype x LED: $F(2,28) = 0.42$, $p = 0.66$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(7)$ ratio = 2.57, $p = 0.0369 \times 2 = 0.074$ Opsin +ve baseline-recovery: $t(7) = 0.42$, $p = 0.687 \times 2 = 1.37$ Opsin -ve baseline-trial: $t(7) = 2.45$, $p = 0.0440 \times 2 = 0.088$ Opsin -ve baseline-recovery: $t(7) = 1.30$, $p = 0.236 \times 2 = 0.47$
		Ciii	Day 1: Parametric one-way repeated ANOVA for opsin +ve and opsin -ve groups with post-hoc Student's T-test with Bonferroni	Day 1: Opsin +ve: LED: $F(3,21) = 1.64$, $p = 0.21$ Opsin -ve: LED: $F(3,21) = 3.19$, $p = 0.05$ Pairwise Contrasts: Opsin -ve baseline-LEDON1: $t(7) = 3.28$, $p = 0.0135 \times 3 = 0.041$ Opsin -ve baseline-LEDON2: $t(7) = 1.58$,

			correction (x3 comparisons) Day 2: Parametric one-way repeated ANOVA for opsin +ve and opsin -ve groups with post-hoc Student's T-test with Bonferroni correction (x3 comparisons)	$p = 0.158$ x 3 = 0.47 Opsin -ve baseline-LEDON3: $t(7) = 2.37$, $p = 0.0494$ x 3 = 0.15 Day2: Opsin +ve: LED: $F(3,21) = 2.56$, $p = 0.082$ Opsin -ve: LED: $F(3,21) = 3.58$, $p = 0.031$ Pairwise Contrasts: Opsin -ve baseline-LEDON1: $t(7) = 2.83$, $p = 0.0255$ x 3 = 0.077 Opsin -ve baseline-LEDON2: $t(7) = 2.16$, $p = 0.0677$ x 3 = 0.20 Opsin -ve baseline-LEDON3: $t(7) = 0.35$, $p = 0.737$ x 3 = 2.21
Figure 6	Opsin +ve: n = 10 Opsin -ve: n = 8	Ai	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,16) = 8.026$, $p = 0.012$ LED: $F(2,32) = 3.47e-03$, $p = 0.10$ Genotype x LED: $F(2,32) = 4.77$, $p = 0.015$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -2.60$, $p = 0.0286$ x 2 = 0.057 Opsin +ve baseline-recovery: $t(9) = -1.67$, $p = 0.129$ x 2 = 0.26 Opsin -ve baseline-trial: $t(7) = 1.64$, $p = 0.145$ x 2 = 0.29 Opsin -ve baseline-recovery: $t(7) = 1.52$, $p = 0.173$ x 2 = 0.35
		Aii	Two-way mixed repeated ANOVA.	Genotype: $F(1,16) = 1.45$, $p = 0.25$ LED: $F(2,32) = 1.70$, $p = 0.20$ Genotype x LED: $F(2,32) = 0.33$, $p = 0.72$
		Aiii	Two-way mixed repeated ANOVA.	Genotype: $F(1,16) = 0.84$, $p = 0.37$ LED: $F(2,32) = 2.23$ $p = 0.12$ Genotype x LED: $F(2,32) = 0.29$, $p = 0.75$
		Aiv	Two-way mixed repeated ANOVA.	Genotype: $F(1,16) = 5.89$, $p = 0.027$ LED: $F(2,32) = 0.29$, $p = 0.75$ Genotype x LED: $F(2,32) = 0.64$, $p = 0.54$
		Bi	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,16) = 0.11$, $p = 0.74$ LED: $F(2,32) = 4.47$, $p = 0.02$ Genotype x LED: $F(2,32) = 1.18$, $p = 0.32$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -2.016$, $p = 0.0747$ x 2 = 0.15 Opsin +ve baseline-recovery: $t(9) = -3.13$, $p = 0.0120$ x 2 = 0.024 Opsin -ve baseline-trial: $t(7) = -1.088$, $p = 0.313$ x 2 = 0.63 Opsin -ve baseline-recovery: $t(7) = -1.27$, $p = 0.245$ x 2 = 0.49
		Bii	Two-way mixed repeated ANOVA.	Genotype: $F(1,16) = 6.75$, $p = 0.019$ LED: $F(2,32) = 0.05$, $p = 0.95$ Genotype x LED: $F(2,32) = 0.89$, $p = 0.42$

		Biii	Two-way mixed repeated ANOVA.	Genotype: $F(1,16) = 7.85$, $p = 0.013$ LED: $F(2,32) = 0.19$, $p = 0.83$ Genotype x LED: $F(2,32) = 0.09$, $p = 0.91$
		Biv	Two-way mixed repeated ANOVA.	Genotype: $F(1,16) = 14.53$, $p = 1.53e-03$ LED: $F(2,32) = 0.30$, $p = 0.74$ Genotype x LED: $F(2,32) = 0.30$, $p = 0.75$
		Ci	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,26) = 4.40$, $p = 0.052$ LED: $F(2,32) = 1.48$, $p = 0.24$ Genotype x LED: $F(2,32) = 4.71$, $p = 0.016$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -2.53$, $p = 0.0325 \times 2 = 0.065$ Opsin +ve baseline-recovery: $t(9) = -2.760$, $p = 0.0443 \times 2 = 0.089$ Opsin -ve baseline-trial: $t(7) = 0.88$, $p = 0.408 \times 2 = 0.82$ Opsin -ve baseline-recovery: $t(7) = 0.80$, $p = 0.450 \times 2 = 0.90$
		Cii	Two-way mixed repeated ANOVA.	Genotype: $F(1,16) = 4.037$, $p = 0.062$ LED: $F(2,32) = 0.95$, $p = 0.40$ Genotype x LED: F(2,32) = 1.01, $p = 0.38$
		Ciii	Two-way mixed repeated ANOVA.	Genotype: $F(1,16) = 4.0$ $p = 0.063$ LED: $F(2,32) = 1.28$, $p = 0.29$ Genotype x LED: $F(2,32) = 0.27$, $p = 0.77$
		Civ	Two-way mixed repeated ANOVA.	Genotype: $F(1,16) = 10.78$, $p = 4.68e-03$ LED: $F(2,32) = 0.43$, $p = 0.66$ Genotype x LED: $F(2,32) = 0.36$, $p = 0.70$
Figure 7	Opsin +ve: n = 10 Opsin -ve: n = 8	Ai	Opsin +ve: Parametric one-way repeated ANOVA with post-hoc Student's T-test with Bonferroni correction (x3 comparisons) Opsin -ve: Non-parametric Friedman's test with post-hoc Wilcoxon Rank Sum test with Bonferroni correction (x3 comparisons)	Opsin +ve: Baseline number: $F(3,21) = 3.52$, $p = 0.029$ Opsin -ve: $\chi^2(3) = 9.45$, $p = 0.024$ Pairwise Contrasts: Opsin +ve baseline vid1-baseline vid2: $t(9) = -2.51$, $p = 0.0332 \times 3 = 0.10$ Opsin +ve baseline vid1-baseline vid3: $t(9) = -2.37$, $p = 0.0416 \times 3 = 0.13$ Opsin +ve baseline vid1-baseline vid4: $t(9) = -1.94$, $p = 0.0841 \times 3 = 0.25$ Opsin -ve baseline vid1-baseline vid2: $V = 30$, $p = 0.109 \times 3 = 0.33$ Opsin -ve baseline vid1-baseline vid3: $V = 34$, $p = 0.0234 \times 3 = 0.07$ Opsin -ve baseline vid1-baseline vid4: $V = 36$, $p = 0.00781 \times 3 = 0.023$
		Bi	Opsin +ve: Non-parametric Friedman's test with post-hoc Wilcoxon Rank Sum test with	Opsin +ve: $\chi^2(3) = 10.44$, $p = 0.015$ Opsin -ve: Baseline number: $F(3,21) = 1.63$, $p = 0.21$

			Bonferroni correction (x3 comparisons) Opsin -ve: Parametric one-way repeated ANOVA with post-hoc Student's T-test with Bonferroni correction (x3 comparisons)	Pairwise Contrasts: Opsin +ve baseline vid1-baseline vid2: *V* = 0, *p* = 0.00195 x 3 = 0.0059 Opsin +ve baseline vid1-baseline vid3: *V* = 7, *p* = 0.0371 x 3 = 0.11 Opsin +ve baseline vid1-baseline vid4: *V* = 6, *p* = 0.0273 x 3 = 0.082
		Ci	Opsin +ve: Non-parametric Friedman's test with post-hoc Wilcoxon Rank Sum test with Bonferroni correction (x3 comparisons) Opsin -ve: Parametric one-way repeated ANOVA with post-hoc Student's T-test with Bonferroni correction (x3 comparisons)	Opsin +ve: $\chi^2(3)$ = 13.8, *p* = 0.0032 Opsin -ve: Baseline number: *F*(3,21) = 5.172, *p* = 0.0078 Pairwise Contrasts: Opsin +ve baseline vid1-baseline vid2: *V* = 0, *p* = 0.00195 x 3 = 0.0059 Opsin +ve baseline vid1-baseline vid2: *V* = 4, *p* = 0.0137 x 3 = 0.041 Opsin +ve baseline vid1-baseline vid3: *V* = 4, *p* = 0.0137 x 3 = 0.041 Opsin -ve baseline vid1-baseline vid2: *t*(7) = 2.43, *p* = 0.0455 x 3 = 0.14 Opsin -ve baseline vid1-baseline vid3: *t*(7) = 2.89, *p* = 0.0235 x 3 = 0.071 Opsin -ve baseline vid1-baseline vid4: *t*(7) = 3.81, *p* = 0.00667 x 3 = 0.02
		Aii	Video 1 and 4 baseline comparisons: Student's T-test	Video 1: *t*(16) = -3.81, *p* = 0.0015 Video 4: *t*(16) = 1.35, *p* = 0.20
		Bii	Video 1 and 4 baseline comparisons: Student's T-test	Video 1: *t*(16)= -0.91, *p* = 0.38 Video 4: *t*(16)= 2.40, *p* = 0.029
		Cii	Video 1 and 4 baseline comparisons: Student's T-test	Video 1: *t*(16)= -4.07, *p* = 0.00089 Video 4: *t*(16)= 2.18, *p* = 0.045
Figure 8	Opsin +ve: n = 10 Opsin -ve: n = 8	Ai	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: *F*(1,16) = 1.21, *p* = 0.29 LED: *F*(2,32) = 1.06, *p* = 0.36 Genotype x LED: *F*(2,32) = 4.44, p = 0.02 Pairwise Contrasts: Opsin +ve baseline-trial: *t*(9) = -3.34, *p* = 0.00870 x 2 = 0.017 Opsin +ve baseline-recovery: *t*(9) = -1.22, *p* = 0.253 x 2 = 0.51 Opsin -ve baseline-trial: *t*(7) = 1.15, *p* = 0.290 x 2 = 0.58

				Opsin -ve baseline-recovery: $t(7) = 1.16$, $p = 0.284 \times 2 = 0.57$
		Bi	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,16) = 0.66$, $p = 0.43$ LED: $F(2,32) = 0.35$, $p = 0.71$ Genotype x LED: $F(2,32) = 3.69$, $p = 0.036$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -2.49$, $p = 0.0345 \times 2 = 0.069$ Opsin +ve baseline-recovery $t(9) = -1.97$, $p = 0.0805 \times 2 = 0.16$ Opsin -ve baseline-trial: $t(7) = 1.10$, $p = 0.306 \times 2 = 0.61$ Opsin -ve baseline-recovery: $t(7) = 1.42$, $p = 0.198 \times 2 = 0.40$
		Ci	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,16) = 4.83$, $p = 0.043$ LED: $F(2,32) = 5.24$, $p = 0.011$ Genotype x LED: $F(2,32) = 3.66$, $p = 0.037$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = 1.35$, $p = 0.211 \times 2 = 0.42$ Opsin +ve baseline-recovery: $t(9) = -1.66$, $p = 0.131 \times 2 = 0.26$ Opsin -ve baseline-trial: $t(7) = 3.049$, $p = 0.0186 \times 2 = 0.037$ Opsin -ve baseline-trial: $t(7) = 1.57$, $p = 0.161 \times 2 = 0.32$
		Di	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 0.75$, $p = 0.40$ LED: $F(2,32) = 1.75$, $p = 0.19$ Genotype x LED: $F(2,32) = 1.29$, $p = 0.29$
		Ei	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,16) = 1.52$, $p = 0.24$ LED: $F(2,32) = 0.14$, $p = 0.87$ Genotype x LED: $F(2,32) = 4.57$, $p = 0.018$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -0.40$, $p = 0.670 \times 2 = 1.34$ Opsin +ve baseline-recovery: $t(9) = -1.73$, $p = 0.117 \times 2 = 0.23$ Opsin -ve baseline-trial: $t(7) = 1.18$, $p = 0.277 \times 2 = 0.55$ Opsin -ve baseline-recovery: $t(7) = 1.73$, $p = 0.127 \times 2 = 0.25$
		Aii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 15.698$, $p = 1.12e-03$ LED: $F(2,32) = 0.39$, $p = 0.68$ Genotype x LED: $F(2,32) = 1.35$, $p = 0.27$
		Bii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 5.54$, $p = 0.032$ LED: $F(2,32) = 0.47$, $p = 0.63$ Genotype:LED: $F(2,32) = 1.75$, $p = 0.19$
		Cii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 7.31$, $p = 0.016$ LED: $F(2,32) = 4.28$, $p = 0.023$

			followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype:LED: $F(2,32) = 0.91$, $p = 0.41$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = 0.53$, $p = 0.611 \times 2 = 1.22$ Opsin +ve baseline-recovery: $t(9) = -1.21$, $p = 0.258 \times 2 = 0.52$ Opsin -ve baseline-trial: $t(7) = 3.71$, $p = 0.00753 \times 2 = 0.015$ Opsin -ve baseline-recovery: $t(7)= 0.21$, $p = 0.844 \times 2 = 1.69$
		Dii	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,16) = 2.19$, $p = 0.16$ LED: $F(2,32) = 4.36$, $p = 0.021$ Genotype:LED: $F(2,32) = 2.16$, $p = 0.13$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = 0.33$, $p = 0.752 \times 2 = 1.50$ Opsin +ve baseline-recovery: $t(9) = -1.30$, $p = 0.227 \times 2 = 0.45$ Opsin -ve baseline-trial: $t(7) = 4.97$, $p = 0.00161 \times 2 = 0.0032$ Opsin +ve baseline-recovery: $t(7) = 0.81$, $p = 0.445 \times 2 = 0.89$
		Eii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 2.14$, $p = 0.16$ LED: $F(2,32) = 0.24$, $p = 0.79$ Genotype x LED: $F(2,32) = 1.71$, $p = 0.20$
Figure 9	Opsin +ve: n = 7 Opsin -ve: n = 6	Ai	Two-way mixed repeated ANOVA	Genotype: $F(1,11) = 0.65$, $p = 0.44$ LED: $F(2,22) = 2.12$, $p = 0.14$ Genotype x LED: $F(2,22) = 1.53$, $p = 0.24$
		Bi	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,11) = 0.087$, $p = 0.77$ LED: $F(2,22) = 9.17$, $p = 1.27e-03$ Genotype:LED: $F(2,22) = 2.23$, $p = 0.13$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(6) = 2.009$, $p = 0.0913 \times 2 = 0.18$ Opsin +ve baseline-recovery: $t(6) = 4.38$, $p = 0.00467 \times 2 = 0.0093$ Opsin -ve baseline-trial: $t(5) = -0.28$, $p = 0.791 \times 2 = 1.58$ Opsin -ve baseline-recovery: $t(5) = 3.088$, $p = 0.0272 \times 2 = 0.054$
		Ci	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,11) = 0.016$, $p = 0.90$ LED: $F(2,22) = 4.59$, $p = 0.022$ Genotype x LED: $F(2,22) = 1.49$, $p = 0.25$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(6) = 4.31$, $p = 0.00501 \times 2 = 0.01$ Opsin +ve baseline-recovery: $t(6) = 3.28$, $p = 0.0168 \times 2 = 0.034$ Opsin -ve baseline-trial: $t(5) = 0.53$,

				p = 0.616 x 2 = 1.23 Opsin -ve baseline-recovery: $t(5)$ = 0.70, p = 0.516 x 2 = 1.032
		Di	Two-way mixed repeated ANOVA	Genotype: F(1,11) = 0.955, p = 0.35 LED: F(2,22) = 2.48, p = 0.11 Genotype x LED: F(2,22) = 0.096, p = 0.91
		Ei	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,11)$ = 1.77, p = 0.21 LED: $F(2,22)$ = 3.64, p = 0.043 Genotype x LED: $F(2,22)$ = 0.67, p = 0.52 Pairwise Contrasts: Opsin +ve baseline-trial: $t(6)$ = 1.27, p = 0.251 x 2 = 0.50 Opsin +ve baseline-recovery: $t(6)$ = 3.77, p = 0.00929 x 2 = 0.019 Opsin -ve baseline-trial: $t(5)$ = -0.22, p = 0.836 x 2 = 1.67 Opsin -ve baseline-recovery: $t(5)$ = 1.20, p = 0.283 x 2 = 0.57
		Aii	Two-way mixed repeated ANOVA	Genotype: $F(1,11)$ = 1.091, p = 0.32 LED: $F(2,22)$ = 1.467, p = 0.25 Genotype x LED: $F(2,22)$ = 0.91, p = 0.42
		Bii	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,11)$ = 0.036, p = 0.85 LED: $F(2,22)$ = 1.74, p = 0.12 Genotype x LED: $F(2,22)$ = 3.52, p = 0.047 Pairwise Contrasts: Opsin +ve baseline-trial: $t(6)$ = -2.53, p = 0.0448 x 2 = 0.09 Opsin +ve baseline-recovery: $t(6)$= 0.84, p = 0.433 x 2 = 0.87 Opsin -ve baseline-trial: $t(5)$ = 0.73, p = 0.497 x 2 = 0.99 Opsin -ve baseline-recovery: $t(5)$= 0.19, p = 0.859 x 2 = 1.72
		Cii	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,11)$ = 0.62, p = 0.45 LED: $F(2,22)$ = 4.292 p = 0.027 Genotype x LED: F(2,22) = 3.30, p = 0.056 Pairwise Contrasts: Opsin +ve baseline-trial: $t(6)$= -0.61, p = 0.566 x 2 = 1.13 Opsin +ve baseline-recovery: $t(6)$= -1.57, p = 0.168 x 2 = 0.34 Opsin -ve baseline-trial: $t(5)$ = 3.74, p = 0.0134 X 2 = 0.027 Opsin -ve baseline-recovery: $t(5)$ = 0.16, p = 0.878 x 2 = 1.76
		Dii	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with	Genotype: $F(1,11)$ = 0.34, p = 0.57 LED: $F(2,22)$ = 4.93, p = 0.017 Genotype x LED: $F(2,22)$ = 1.83, p = 0.18 Pairwise Contrasts:

			Bonferroni correction (x2 comparisons).	Opsin +ve baseline-trial: $t(6) = 0.30$, $p = 0.771 \times 2 = 1.54$ Opsin +ve baseline-recovery: $t(6) = -0.75$, $p = 0.479 \times 2 = 0.96$ Opsin -ve baseline-trial: $t(5) = 2.21$, $p = 0.0786 \times 2 = 0.16$ Opsin -ve baseline-trial: $t(5) = -0.66$, $p = 0.540 \times 2 = 1.08$
		Eii	Two-way mixed repeated ANOVA	Genotype: $F(1,11) = 0.39$, $p = 0.54$ LED: $F(2,22) = 0.62$, $p = 0.55$ Genotype x LED: $F(2,22) = 1.18$, $p = 0.33$
Figure 10	Opsin +ve: n = 10 Opsin -ve: n = 8	Ai	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 1.96$, $p = 0.18$ LED: $F(2,32) = 0.05$, $p = 0.95$ Genotype x LED: $F(2,32) = 1.32$, $p = 0.28$
		Aii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 1.063$, $p = 0.32$ LED: $F(2,32) = 2.45$, $p = 0.10$ Genotype x LED: $F(2,32) = 2.76$, $p = 0.079$
		Aiii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 1.46$, $p = 0.25$ LED: $F(2,32) = 0.45$, p = 0.64 Genotype x LED: $F(2,32) = 1.22$, $p = 0.31$
		Aiv	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 3.64$, $p = 0.075$ LED: $F(2,32) = 0.094$, $p = 0.91$ Genotype x LED: $F(2,32) = 0.22$, $p = 0.80$
		Bi	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 0.42$, $p = 0.53$ LED: $F(2,32) = 1.88$, $p = 0.17$ Genotype x LED: $F(2,23) = 2.98$, $p = 0.065$
		Bii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 9.40$, $p = 7.38e-03$ LED: $F(2,32) = 0.12$, $p = 0.88$ Genotype x LED: $F(2,32) = 0.35$, $p = 0.71$
		Biii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 20.26$, $p = 3.63e-04$ LED: $F(2,32) = 0.24$, $p = 0.79$ Genotype x LED: $F(2,32) = 1.27$, $p = 0.29$
		Biv	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 18.79$, $p = 5.12e-04$ LED: $F(2,32) = 0.55$, $p = 0.58$ Genotype x LED: $F(2,32) = 0.086$, $p = 0.92$
		Ci	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons).	Genotype: $F(1,16) = 2.41$, $p = 0.14$ LED: $F(2,32) = 1.24$, $p = 0.30$ Genotype x LED: $F(2,32) = 4.55$, $p = 0.018$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -2.49$, $p = 0.0342 \times 2 = 0.068$ Opsin +ve baseline-recovery: $t(9) = -2.40$, $p = 0.0402 \times 2 = 0.080$ Opsin -ve baseline-trial: $t(7) = 1.71$, $p = 0.131 \times 2 = 0.26$ Opsin -ve baseline-recovery: $t(7) = 0.61$, $p = 0.562 \times 2 = 1.12$
		Cii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 5.165$, $p = 0.037$ LED: $F(2,32) = 1.375$, $p = 0.27$ Genotype x LED: $F(2,32) = 1.60$, $p = 0.22$
		Ciii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 7.36$, $p = 0.015$ LED: $F(2,32) = 0.50$, $p = 0.61$ Genotype x LED: $F(2,32) = 1.10$, $p = 0.35$

		Civ	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 9.82$, $p = 6.41e\text{-}03$ LED: $F(2,32) = 0.45$, $p = 0.64$ Genotype x LED: $F(2,32) = 0.18$, $p = 0.84$
Figure 11	Opsin +ve: n = 10 Opsin -ve: n = 8	Ai	Opsin +ve: Non-parametric Friedman's test. Opsin -ve: Parametric one-way repeated ANOVA with post-hoc Student's T-test with Bonferroni correction (x3 comparisons)	Opsin +ve: $\chi^2(3) = 1.18$, $p = 0.76$ Opsin -ve: Baseline number: $F(3,21) = 3.98$, $p = 0.022$ Pairwise Contrasts: Opsin -ve baseline vid1-baseline vid2: $t(7) = 0.13$, $p = 0.897 \times 3 = 2.69$ Opsin -ve baseline vid1-baseline vid3: $t(7) = 1.43$, $p = 0.195 \times 3 = 0.59$ Opsin -ve baseline vid1-baseline vid4: $t(7) = 4.23$, $p = 0.00387 \times 3 = 0.012$
		Aii	Video 1 and 4: Student's T-test	Video 1: $t(16) = -2.12$, $p = 0.05$ Video 4: $t(16) = 1.072$, $p = 0.3$
		Bi	Opsin +ve: Parametric one-way repeated ANOVA Opsin -ve: Parametric one-way repeated ANOVA with post-hoc Student's T-test with Bonferroni correction (x3 comparisons)	Opsin +ve: Baseline number: $F(3,27) = 2.43$, $p = 0.087$ Opsin -ve: Baseline number: $F(3,27) = 3.51$, $p = 0.033$ Pairwise Contrasts: Opsin -ve baseline vid1-baseline vid2: $t(7) = 2.76$, $p = 0.0283 \times 3 = 0.085$ Opsin -ve baseline vid1-baseline vid3: $t(7) = 3.16$, $p = 0.0158 \times 3 = 0.048$ Opsin -ve baseline vid1-baseline vid4: $t(7) = 3.43$, $p = 0.0109 \times 3 = 0.033$
		Bii	Video 1 and 4: Student's T-test	Video 1: $t(16) = -2.23$, $p = 0.040$ Video 4: $t(16) = 2.0$, $p = 0.063$
		Ci	Opsin +ve: Non-parametric Friedman's test with post-hoc Wilcoxon Rank-Sum test with Bonferroni correction (x3 comparisons) Opsin -ve: Parametric one-way repeated ANOVA with post-hoc Student's T-test with Bonferroni correction (x3 comparisons)	Opsin +ve: $\chi^2(3) = 9.44$, $p = 0.024$ Opsin -ve: Baseline number: $F(3,21) = 5.95$, $p = 0.0042$ Pairwise Contrasts: Opsin +ve baseline vid1-baseline vid2: $V = 4$, $p = 0.0137 \times 3 = 0.041$ Opsin +ve baseline vid1-baseline vid3: $V = 7$, $p = 0.0371 \times 3 = 0.11$ Opsin +ve baseline vid1-baseline vid4: $V = 6.5$, $p = 0.0364 \times 3 = 0.11$ Opsin -ve baseline vid1-baseline vid2: $t(7) = 3.38$, $p = 0.0119 \times 3 = 0.036$ Opsin -ve baseline vid1-baseline vid3: $t(7) = 2.66$, $p = 0.0323 \times 3 = 0.097$ Opsin -ve baseline vid1-baseline vid4: $t(7) = 4.36$, $p = 0.00334 \times 3 = 0.01$

		Cii	Video 1: Wilcoxon Rank Sum Test	Video 1: $W = 7.5$, $p = 0.0043$ Video 4: $t(16) = 1.91$, $p = 0.074$
Figure 12	Opsin +ve: n = 10 Opsin -ve: n = 8	Ai	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons)	Genotype: $F(1,16) = 0.44$, $p = 0.52$ LED: $F(2,32) = 6.14$, $p = 5.53\text{e-}03$ Genotype x LED: $F(2,32) = 2.83$, $p = 0.074$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -3.48$, $p = 0.0069 \times 2 = 0.014$ Opsin +ve baseline-recovery: $t(9) = -2.48$, $p = 0.0353 \times 2 = 0.071$ Opsin -ve baseline-trial: $t(7) = -0.97$, $p = 0.362 \times 2 = 0.72$ Opsin -ve baseline-recovery: $t(7) = 0.066$, $p = 0.949 \times 2 = 1.90$
		Aii	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons)	Genotype: $F(1,16) = 4.0$, $p = 0.063$ LED: $F(2,32) = 3.45$, $p = 0.044$ Genotype x LED: $F(2,32) = 1.10$, $p = 0.35$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -2.36$, $p = 0.0423 \times 2 = 0.085$ Opsin +ve baseline-recovery: $t(9) = -2.33$, $p = 0.0445 \times 2 = 0.089$ Opsin -ve baseline-trial: $t(7) = -0.433$, $p = 0.678 \times 2 = 1.36$ Opsin -ve baseline-recovery: $t(7) = -0.95$, $p = 0.374 \times 2 = 0.75$
		Bi	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons)	Genotype: F(1,16) = 1.10, p = 0.31 LED: F(2,32) = 0.41, p = 0.67 Genotype x LED: F(2,32) = 3.81, p = 0.033 Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -3.073$, $p = 0.0133 \times 2 = 0.027$ Opsin +ve baseline-recovery: $t(9) = -2.74$, $p = 0.0227 \times 2 = 0.045$ Opsin -ve baseline-trial: $t(7) = 0.69$, $p = 0.511 \times 2 = 1.022$ Opsin -ve baseline-recovery: $t(7) = 1.78$, $p = 0.119 \times 2 = 0.24$
		Bii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 2.67$, $p = 0.12$ LED: $F(2,32) = 0.35$, $p = 0.71$ Genotype x LED: $F(2,32) = 2.26$, $p = 0.12$
		Ci	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons)	Genotype: $F(1,16) = 0.61$, $p = 0.45$ LED: $F(2,32) = 4.16$, $p = 0.025$ Genotype x LED: $F(2,32) = 0.51$, $p = 0.61$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -2.80$, $p = 0.0206 \times 2 = 0.041$

				Opsin +ve baseline-recovery: $t(9) = -1.57$, $p = 0.151 \times 2 = 0.30$ Opsin -ve baseline-trial: $t(7) = -1.58$, $p = 0.160 \times 2 = 0.32$ Opsin -ve baseline-recovery: $t(7) = -1.66$, $p = 0.142 \times 2 = 0.28$
		Cii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 1.53$, $p = 0.24$ LED: $F(2,32) = 2.54$, $p = 0.095$ Genotype x LED: $F(2,32) = 0.43$, $p = 0.65$
		Di	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons)	Genotype: $F(1,16) = 0.61$, $p = 0.45$ LED: $F(2,32) = 4.16$, $p = 0.025$ Genotype x LED: $F(2,32) = 0.51$, $p = 0.61$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -4.55$, $p = 0.00139 \times 2 = 0.0028$ Opsin +ve baseline-recovery: $t(9) = -2.75$, $p = 0.0223 \times 2 = 0.045$ Opsin -ve baseline-trial: $t(7) = 0.37$, $p = 0.724 \times 2 = 1.45$ Opsin -ve baseline-recovery: $t(7) = 0.56$, $p = 0.591 \times 2 = 1.18$
		Dii	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 1.53$, $p = 0.24$ LED: $F(2,32) = 2.54$, $p = 0.095$ Genotype x LED: $F(2,32) = 0.43$, $p = 0.65$
		Ei	Two-way mixed repeated ANOVA	Genotype: $F(1,16) = 2.23$, $p = 0.16$ LED: $F(2,32) = 1.22$, $p = 0.31$ Genotype x LED: $F(2,32) = 1.24$, $p = 0.30$
		Eii	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons)	Genotype: $F(1,16) = 0.15$, $p = 0.70$ LED: $F(2,32) = 3.29$, $p = 0.05$ Genotype x LED: $F(2,32) = 4.31$, $p = 0.022$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(9) = -1.49$, $p = 0.170 \times 2 = 0.34$ Opsin +ve baseline-recovery: $t(9) = -0.11$, $p = 0.915 \times 2 = 1.83$ Opsin -ve baseline-trial: $t(7) = -0.93$, $p = 0.384 \times 2 = 0.77$ Opsin -ve baseline-recovery: $t(7) = -3.67$, $p = 0.00794 \times 2 = 0.016$
Figure 13	Opsin +ve: n = 7 Opsin -ve: n = 6	Ai	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons)	Genotype: $F(1,11) = 0.075$, $p = 0.79$ LED: $F(2,22) = 2.67$, $p = 0.092$ Genotype x LED: $F(2,22) = 5.15$, $p = 0.015$ Pairwise Contrasts: Opsin +ve baseline-trial: $t(6) = -0.11$, $p = 0.913 \times 2 = 1.83$ Opsin +ve baseline-recovery: $t(6) = -0.70$, $p = 0.512 \times 2 = 1.024$ Opsin -ve baseline-trial: $t(5) = -1.63$, $p = 0.164 \times 2 = 0.33$ Opsin -ve baseline-recovery: $t(5) = 2.53$,

				p = 0.0523 x 2 = 0.11
		Aii	Two-way mixed repeated ANOVA	Genotype: $F(1,11) = 0.51$, $p = 0.49$ LED: $F(2,22) = 1.59$, $p = 0.23$ Genotype x LED: $F(2,22) = 0.031$, $p = 0.97$
		Bi	Two-way mixed repeated ANOVA followed by post-hoc Student's T-test with Bonferroni correction (x2 comparisons)	Genotype: $F(1,11) = 0.32$, $p = 0.58$ LED: $F(2,22) = 3.36$, $p = 0.053$ Genotype x LED: $F(2,22) = 1.83$, $p = 0.18$ Pairwise Contrast: Opsin +ve baseline-trial: $t(6) = 0.22$, p = 0.834 x 2 = 1.67 Opsin +ve baseline-recovery: $t(6)= 0.69$, p = 0.516 x 2 = 1.032 Opsin -ve baseline-trial: $t(5) = -0.10$, p = 0.922 x 2 = 1.84 Opsin -ve baseline-recovery: $t(5) = 3.0$, p = 0.0305 x 2 = 0.061
		Bii	Two-way mixed repeated ANOVA	Genotype: $F(1,11) = 0.115$, $p = 0.74$ LED: $F(2,22) = 0.067$, $p = 0.94$ Genotype x LED: $F(2,22) = 2.085$, $p = 0.15$
		Ci	Two-way mixed repeated ANOVA	Genotype: $F(1,11) = 0.87$, $p = 0.37$ LED: $F(2,22) = 2.15$, $p = 0.14$ Genotype x LED: $F(2,22) = 1.54$, $p = 0.24$
		Cii	Two-way mixed repeated ANOVA	Genotype: $F(1,11) = 1.37$, $p = 0.27$ LED: $F(2,22) = 2.50$, $p = 0.10$ Genotype x LED: $F(2,22) = 0.19$, $p = 0.83$
		Di	Two-way mixed repeated ANOVA	Genotype: $F(1,11) = 0.87$, $p = 0.37$ LED: $F(2,22) = 2.15$, $p = 0.14$ Genotype x LED: $F(2,22) = 1.54$, $p = 0.24$
		Dii	Two-way mixed repeated ANOVA	Genotype: $F(1,11) = 0.74$, $p = 0.41$ LED: $F(2,22) = 0.98$, $p = 0.39$ Genotype x LED: $F(2,22) = 1.063$, $p = 0.36$
		Ei	Two-way mixed repeated ANOVA	Genotype: $F(1,11) = 0.003$, $p = 0.96$ LED: $F(2,22) = 0.36$, $p = 0.71$ Genotype x LED: $F(2,22) = 0.18$, $p = 0.84$
		Eii	Two-way mixed repeated ANOVA	Genotype: $F(1,11) = 0.34$, $p = 0.57$ LED: $F(2,22) = 0.01$, $p = 0.99$ Genotype x LED: $F(2,22) = 2.28$, $p = 0.13$

Albin, R. L., Young, A. B., and Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. Trends in Neurosciences. *12*(10), 366-375.

Alexander, G. E., DeLong, M. R., and Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annual Review of Neuroscience. *9*(1), 357-381.

Alexander, G. E., and Crutcher, M. D. (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends in Neurosciences. *13*(7), 266-271.

Alexander, G. E. (1994). Basal ganglia-thalamocortical circuits: their role in control of movements. Journal of Clinical Neurophysiology. *11*(4), 420-431.

Anwyl, R. (2009). Metabotropic glutamate receptor-dependent long-term potentiation. Neuropharmacology. *56*(4), 735-740.

Apicella, P., Ljungberg, T., Scarnati, E., and Schultz, W. (1991). Responses to reward in monkey dorsal and ventral striatum. Experimental Brain research. *85*, 491-500.

Atallah, H. E., Lopez-Paniagua, D., Rudy, J. W., and O'Reilly, R. C. (2007). Separate neural substrates for skill learning and performance in the ventral and dorsal striatum. Nature Neuroscience. *10*(1), 126-131.

Bäckman, C. M., Malik, N., Zhang, Y., Shan, L., Grinberg, A., Hoffer, B. J., Westphal, H., and Tomac, A. C. (2006). Characterization of a mouse strain expressing Cre recombinase from the 3′ untranslated region of the dopamine transporter locus. Genesis. *44*(8), 383-390.

Bailey, K. R., and Mair, R. G. (2006). The role of striatum in initiation and execution of learned action sequences in rats. Journal of Neuroscience. *26*(3), 1016-1025.

Bardo, M. T., Donohew, R. L., & Harrington, N. G. (1996). Psychobiology of novelty seeking and drug seeking behavior. Behavioural Brain Research. *77*(1-2), 23-43.

Barter, J. W., Li, S., Lu, D., Bartholomew, R. A., Rossi, M. A., Shoemaker, C. T., Salas-Meza, D., Gaidis, E., and Yin, H. H. (2015). Beyond reward prediction errors: the role of dopamine in movement kinematics. Frontiers in Integrative Neuroscience. *9*, 39.

Bartholomew, R. A., Li, H., Gaidis, E. J., Stackmann, M., Shoemaker, C. T., Rossi, M. A., and Yin, H. H. (2016). Striatonigral control of movement velocity in mice. European Journal of Neuroscience. *43*(8), 1097-1110.

Berardelli, A., Rothwell, J. C., Thompson, P. D., and Hallett, M. (2001). Pathophysiology of

bradykinesia in Parkinson's disease. Brain. *124*(11), 2131-2146.

Berry, A., Bellisario, V., Capoccia, S., Tirassa, P., Calza, A., Alleva, E., and Cirulli, F. (2012). Social deprivation stress is a triggering factor for the emergence of anxiety-and depression-like behaviours and leads to reduced brain BDNF levels in C57BL/6J mice. *Psychoneuroendocrinology*. 37(6), 762-772.

Bissonette, G. B., and Roesch, M. R. (2015). Rule encoding in dorsal striatum impacts action selection. European Journal of Neuroscience. *42*(8), 2555-2567.

Blanchard, V., Raisman-Vozari, R., Vyas, S., Michel, P. P., Javoy-Agid, F., Uhl, G., and Agid, Y. (1994). Differential expression of tyrosine hydroxylase and membrane dopamine transporter genes in subpopulations of dopaminergic neurons of the rat mesencephalon. Molecular Brain Research. *22*(1-4), 29-38.

Brischoux, F., Chakraborty, S., Brierley, D. I., and 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

Bódi, N., Kéri, S., Nagy, H., Moustafa, A., Myers, C. E., Daw, N., Dibó, G., Takats, A., Bereczki, D., and Gluck, M. A. (2009). Reward-learning and the novelty-seeking personality: a between-and within-subjects study of the effects of dopamine agonists on young Parkinson's patients. Brain. *132*(9), 2385-2395.

Bova, A., Gaidica, M., Hurst, A., Iwai, Y., Hunter, J., and Leventhal, D. K. (2020). Precisely timed dopamine signals establish distinct kinematic representations of skilled movements. Elife, *9*. DOI:10.7554/eLife.61591.

Broussard, J. I., Yang, K., Levine, A. T., Tsetsenis, T., Jenson, D., Cao, F., Garcia, I., Arenkiel, B. R., Zhou, F., De Biasi, M., and Dani, J. A. (2016). Dopamine regulates aversive contextual learning and associated in vivo synaptic plasticity in the hippocampus. Cell Reports. *14*(8), 1930-1939.

Carboni, E., Tanda, G. L., Frau, R., and Chiara, G. D. (1990). Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: evidence that dopamine is taken up in vivo by noradrenergic terminals. Journal of Neurochemistry. *55*(3), 1067-1070.

Centonze, D., Usiello, A., Gubellini, P., Pisani, A., Borrelli, E., Bernardi, G., and Calabresi, P. (2002). Dopamine D2 receptor-mediated inhibition of dopaminergic neurons in mice lacking D2L receptors. Neuropsychopharmacology. *27*(5), 723-726.

Cepeda, C., Buchwald, N. A., and Levine, M. S. (1993). Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. Proceedings of the National Academy of Sciences. *90*(20), 9576-9580.

Cepeda, C., Colwell, C. S., Itri, J. N., Chandler, S. H., and Levine, M. S. (1998). Dopaminergic modulation of NMDA-induced whole cell currents in neostriatal neurons in slices: contribution of calcium conductances. Journal of Neurophysiology. *79*(1), 82-94.

Cepeda, C., Murphy, K. P., Parent, M., and Levine, M. S. (2014). The role of dopamine in Huntington's disease. Progress in Brain Research. *211*, 235-254.

Chaudhury, D., Walsh, J. J., Friedman, A. K., Juarez, B., Ku, S. M., Koo, J. W., Ferguson, D., Tsai, H., Pomeranz, L., Christoffel, D. J., Nectow, A. R., Ekstrand, M., Domingos, A., Mazei-Robison, M. S., Mouzon, E., Lobo, M. K., Neve, R. L., Freidman, J. M., Russo, S. J., Deisseroth, K., Nestler, E. J., and Han, M. H. (2013). Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature. *493*(7433), 532-536.

Chu, Y., Kompoliti, K., Cochran, E. J., Mufson, E. J., and Kordower, J. H. (2002). Age-related decreases in Nurr1 immunoreactivity in the human substantia nigra. Journal of Comparative Neurology. *450*(3), 203-214.

Chu, H. Y., and Zhen, X. (2010). Hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels in the regulation of midbrain dopamine systems. Acta Pharmacologica Sinica. *31*(9), 1036-1043.

Costa, V. D., Tran, V. L., Turchi, J., and Averbeck, B. B. (2014). Dopamine modulates novelty seeking behavior during decision making. Behavioral neuroscience. *128*(5), 556.

Cui, G., Jun, S. B., Jin, X., Pham, M. D., Vogel, S. S., Lovinger, D. M., and Costa, R. M. (2013). Concurrent activation of striatal direct and indirect pathways during action initiation. Nature. *494*(7436), 238-242.

Darvas, M., and Palmiter, R. D. (2009). Restriction of dopamine signaling to the dorsolateral striatum is sufficient for many cognitive behaviors. Proceedings of the National Academy of Sciences. *106*(34), 14664-14669.

Da Silva, J. A., Tecuapetla, F., Paixão, V., and Costa, R. M. (2018). Dopamine neuron activity before action initiation gates and invigorates future movements. Nature. *554*(7691), 244-248.

De Leonibus, E., Oliverio, A., and Mele, A. (2005). A study on the role of the dorsal striatum and the nucleus accumbens in allocentric and egocentric spatial memory consolidation. Learning & Memory. *12*(5), 491-503.

Diao, Z., Yao, L., Cheng, Q., Wu, M., Di, Y., Qian, Z., Wei, C., Liu, Y., Tian, Y., and Ren, W. (2021). Involvement of midbrain dopamine neuron activity in negative reinforcement learning in mice. Molecular Neurobiology. *58*(11), 5667-5681.

Diederen, K. M., and Fletcher, P. C. (2021). Dopamine, prediction error and beyond. The

Neuroscientist. *27*(1), 30-46.

Doeller, C. F., King, J. A., and Burgess, N. (2008). Parallel striatal and hippocampal systems for landmarks and boundaries in spatial memory. Proceedings of the National Academy of Sciences, 105(15), 5915-5920.

Dudman, J. T., and Krakauer, J. W. (2016). The basal ganglia: from motor commands to the control of vigor. Current Opinion in Neurobiology. *37*, 158-166.

Ekstrand, M. I., Terzioglu, M., Galter, D., Zhu, S., Hofstetter, C., Lindqvist, E., Thams, S., Bergstrand, A., Hansson, F. S., Trifunovic, A., Hoffer, B., Cullheim, S., Mohammed, A. H., Olson, L., and Larsson, N. G. (2007). Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. Proceedings of the National Academy of Sciences. *104*(4), 1325-1330.

English, D. F., Ibanez-Sandoval, O., Stark, E., Tecuapetla, F., Buzsáki, G., Deisseroth, K., Tepper, J. M., and Koos, T. (2012). GABAergic circuits mediate the reinforcement-related signals of striatal cholinergic interneurons. Nature Neuroscience. *15*(1), 123-130.

Estakhr, J., Abazari, D., Frisby, K., McIntosh, J. M., and Nashmi, R. (2017). Differential control of dopaminergic excitability and locomotion by cholinergic inputs in mouse substantia nigra. Current Biology. *27*(13), 1900-1914.

Evans, R. C., Zhu, M., and Khaliq, Z. M. (2017). Dopamine inhibition differentially controls excitability of substantia nigra dopamine neuron subpopulations through T-type calcium channels. Journal of Neuroscience. *37*(13), 3704-3720.

Fiorillo, C. D., Tobler, P. N., and Schultz, W. (2003). Discrete coding of reward probability and uncertainty by dopamine neurons. Science. 299(5614), 1898-1902.

Flores-Hernandez, J., Cepeda, C., Hernandez-Echeagaray, E., Calvert, C. R., Jokel, E. S., Fienberg, A. A., Greengard, P., and Levine, M. S. (2002). Dopamine enhancement of NMDA currents in dissociated medium-sized striatal neurons: role of D1 receptors and DARPP-32. Journal of Neurophysiology. *88*(6), 3010-3020

Friend, D. M., and Kravitz, A. V. (2014). Working together: basal ganglia pathways in action selection. Trends in Neurosciences. *37*(6), 301-303.

Fu, Y., Yuan, Y., Halliday, G., Rusznák, Z., Watson, C., and Paxinos, G. (2012). A cytoarchitectonic and chemoarchitectonic analysis of the dopamine cell groups in the substantia nigra, ventral tegmental area, and retrorubral field in the mouse. Brain Structure and Function. *217*, 591-612.

Gainetdinov, R. R., Wetsel, W. C., Jones, S. R., Levin, E. D., Jaber, M., and Caron, M. G. (1999). Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science. *283*(5400), 397-401.

Galarraga, E., Herná-López, S., Reyes, A., Barral, J., and Bargas, J. (1997). Dopamine facilitates striatal EPSPs through an L-type Ca2+ conductance. Neuroreport. *8*(9), 2183-2186.

Gantz, S. C., Ford, C. P., Morikawa, H., and Williams, J. T. (2018). The evolving understanding of dopamine neurons in the substantia nigra and ventral tegmental area. Annual Review of Physiology. *80*, 219-241.

Gentet, L. J., and Williams, S. R. (2007). Dopamine gates action potential backpropagation in midbrain dopaminergic neurons. Journal of Neuroscience. *27*(8), 1892-1901.

Gerfen, C. R. (1988). Synaptic organization of the striatum. Journal of Electron Microscopy Technique. *10*(3), 265-281.

German, D. C., and Manaye, K. F. (1993). Midbrain dopaminergic neurons (nuclei A8, A9, and A10): three-dimensional reconstruction in the rat. Journal of Comparative Neurology. *331*(3), 297-309.

Giménez-Llort, L., Martínez, E., and Ferré, S. (1997). Different effects of dopamine antagonists on spontaneous and NMDA-induced motor activity in mice. Pharmacology Biochemistry and Behavior. *56*(3), 549-553.

Giros, B., Jaber, M., Jones, S. R., Wightman, R. M., and Caron, M. G. (1996). Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature. *379*(6566), 606-612.

Goldberg, J. H., Farries, M. A., and Fee, M. S. (2013). Basal ganglia output to the thalamus: still a paradox. Trends in Neurosciences. *36*(12), 695-705.

Gould, T.D., Dao, D.T., and Kovacsics, C.E. (2009). The Open Field Test. In: Gould, T. (eds) Mood and Anxiety Related Phenotypes in Mice. Neuromethods, vol 42. Humana Press, Totowa, NJ.

Grace, A. A., and Bunney, B. S. (1984). The control of firing pattern in nigral dopamine neurons: single spike firing. Journal of Neuroscience. *4*(11), 2866-2876.

Grace, A. A., and Bunney, B. S. (1984). The control of firing pattern in nigral dopamine neurons: burst firing. Journal of Neuroscience. *4*(11), 2877-2890.

Graybiel, A. M. (2000). The basal ganglia. Current Biology. *10*(14), R509-R511.

Graybiel, A. M. (2005). The basal ganglia: learning new tricks and loving it. Current Opinion in Neurobiology. *15*(6), 638-644.

Graybiel, A. M. (2008). Habits, rituals, and the evaluative brain. Annu. Rev. Neurosci. *31*, 359-387.

Graybiel, A. M., and Grafton, S. T. (2015). The striatum: where skills and habits meet. Cold Spring Harbor Perspectives in Biology. *7*(8). DOI: 10.1101/cshperspect.a02169.

Graybiel, A. M., and Matsushima, A. (2020). The ups and downs of the striatum: dopamine biases upstate balance of striosomes and matrix. Neuron. *108*(6), 1013-1015.

Grenhoff, J., Aston-Jones, G., and Svensson, T. H. (1986). Nicotinic effects on the firing pattern of midbrain dopamine neurons. Acta Physiologica Scandinavica. *128*(3), 351-358.

Guo, Q., Wang, D., He, X., Feng, Q., Lin, R., Xu, F., Fu, L., and Luo, M. (2015). Whole-brain mapping of inputs to projection neurons and cholinergic interneurons in the dorsal striatum. PloS One. *10*(4). DOI: 10.1371/journal.pone.0123381.

Hall, C. S. (1934). Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. Journal of Comparative Psychology. *18*(3), 385.

Hallett, P. J., Spoelgen, R., Hyman, B. T., Standaert, D. G., and Dunah, A. W. (2006). Dopamine D1 activation potentiates striatal NMDA receptors by tyrosine phosphorylation-dependent subunit trafficking. Journal of Neuroscience. *26*(17), 4690-4700.

Hamid, A. A., Pettibone, J. R., Mabrouk, O. S., Hetrick, V. L., Schmidt, R., Vander Weele, C. M., Kennedy, R. T., Aragona, B. J., and Berke, J. D. (2016). Mesolimbic dopamine signals the value of work. Nature Neuroscience. *19*(1), 117-126.

Hazy, T. E., Frank, M. J., and O'Reilly, R. C. (2010). Neural mechanisms of acquired phasic dopamine responses in learning. Neuroscience & Biobehavioral Reviews. *34*(5), 701-720.

Henny, P., Brown, M. T., Northrop, A., Faunes, M., Ungless, M. A., Magill, P. J., and Bolam, J. P. (2012). Structural correlates of heterogeneous in vivo activity of midbrain dopaminergic neurons. Nature Neuroscience. *15*(4), 613-619.

Hernandez, P. J., Sadeghian, K., and Kelley, A. E. (2002). Early consolidation of instrumental learning requires protein synthesis in the nucleus accumbens. Nature Neuroscience. *5*(12), 1327-1331.

Hernandez-Lopez, S., Tkatch, T., Perez-Garci, E., Galarraga, E., Bargas, J., Hamm, H., and Surmeier, D. J. (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+ currents and excitability via a novel PLCβ1–IP3–calcineurin-signaling cascade. Journal of Neuroscience. *20*(24), 8987-8995.

Hersch, S. M., Ciliax, B. J., Gutekunst, C. A., Rees, H. D., Heilman, C. J., Yung, K. K., Bolam, J. P., Yi, H., and Levey, A. I. (1995). Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. Journal of Neuroscience. *15*(7), 5222-5237.

Hikima, T., Lee, C. R., Witkovsky, P., Chesler, J., Ichtchenko, K., and Rice, M. E. (2021).

Activity-dependent somatodendritic dopamine release in the substantia nigra autoinhibits the releasing neuron. Cell reports. *35*(1). DOI: 10.1016/j.celrep.2021.108951.

Horvitz, J. C. (2000). Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. Neuroscience. *96*(4), 651-656.

Howe, M. W., and Dombeck, D. A. (2016). Rapid signalling in distinct dopaminergic axons during locomotion and reward. Nature. *535*(7613), 505-510.

Jimenez-Castellanos, J., and Graybiel, A. M. (1987). Subdivisions of the dopamine-containing A8-A9-A10 complex identified by their differential mesostriatal innervation of striosomes and extrastriosomal matrix. Neuroscience. *23*(1), 223-242.

Jin, X., and Costa, R. M. (2010). Start/stop signals emerge in nigrostriatal circuits during sequence learning. Nature. *466*(7305), 457-462.

Jin, M., Min, C., Zheng, M., Cho, D. I., Cheong, S. J., Kurose, H., and Kim, K. M. (2013). Multiple signaling routes involved in the regulation of adenylyl cyclase and extracellular regulated kinase by dopamine D2 and D3 receptors. Pharmacological Research. *67*(1), 31-41.

Jucaite, A., Fernell, E., Halldin, C., Forssberg, H., and Farde, L. (2005). Reduced midbrain dopamine transporter binding in male adolescents with attention-deficit/hyperactivity disorder: association between striatal dopamine markers and motor hyperactivity. Biological Psychiatry. *57*(3), 229-238.

Kakade, S., and Dayan, P. (2002). Dopamine: generalization and bonuses. Neural Networks. *15*(4-6), 549-555.

Kawaguchi, Y., Wilson, C. J., Augood, S. J., and Emson, P. C. (1995). Striatal interneurones: chemical, physiological and morphological characterization. Trends in Neurosciences. *18*(12), 527-535.

Kawaguchi, Y., Aosaki, T., and Kubota, Y. (1997). Cholinergic and GABAergic interneurons in the striatum. Japanese Journal of Psychopharmacology. *17*(2), 87-90.

Keiflin, R., Pribut, H. J., Shah, N. B., and Janak, P. H. (2019). Ventral tegmental dopamine neurons participate in reward identity predictions. Current Biology. *29*(1), 93-103.

Kerr, J. N., and Wickens, J. R. (2001). Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum in vitro. Journal of Neurophysiology. *85*(1), 117-124.

Klaus, A., Alves da Silva, J., and Costa, R. M. (2019). What, if, and when to move: basal ganglia circuits and self-paced action initiation. Annual Review of Neuroscience. *42*, 459-483.

Kravitz, A. V., Freeze, B. S., Parker, P. R., Kay, K., Thwin, M. T., Deisseroth, K., and Kreitzer, A. C. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. Nature. *466*(7306), 622-626.

Krebs, R. M., Heipertz, D., Schuetze, H., and Duzel, E. (2011). Novelty increases the mesolimbic functional connectivity of the substantia nigra/ventral tegmental area (SN/VTA) during reward anticipation: Evidence from high-resolution fMRI. Neuroimage. *58*(2), 647-655.

Kreitzer, A. C., and Malenka, R. C. (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature. *445*(7128), 643-647.

Lanciego, J. L., Luquin, N., and Obeso, J. A. (2012). Functional neuroanatomy of the basal ganglia. Cold Spring Harbor Perspectives in Medicine. *2*(12). DOI: 10.1101/cshperspect.a009621.

Leach, A. C., Pitts, E. G., Siciliano, C. A., and Ferris, M. J. (2022). α7 nicotinic acetylcholine receptor modulation of accumbal dopamine release covaries with novelty seeking. European Journal of Neuroscience. *55*(5), 1162-1173.

Lee, R. S., Mattar, M. G., Parker, N. F., Witten, I. B., and Daw, N. D. (2019). Reward prediction error does not explain movement selectivity in DMS-projecting dopamine neurons. Elife, 8, e42992.

Leo, D., Sorrentino, E., Volpicelli, F., Eyman, M., Greco, D., Viggiano, D., di Porzio, U., and Perrone-Capano, C. (2003). Altered midbrain dopaminergic neurotransmission during development in an animal model of ADHD. Neuroscience & Biobehavioral Reviews. *27*(7), 661-669.

Lerner, T. N., Shilyansky, C., Davidson, T. J., Evans, K. E., Beier, K. T., Zalocusky, K. A., Crow, A. K., Malenka, R. C., Luo, L., Tomer, R., and Deisseroth, K. (2015). Intact-brain analyses reveal distinct information carried by SNc dopamine subcircuits. Cell. *162*(3), 635-647.

Lerner, T. N., Holloway, A. L., and Seiler, J. L. (2021). Dopamine, updated: reward prediction error and beyond. Current Opinion in Neurobiology. *67*, 123-130.

Leussis, M. P., and Bolivar, V. J. (2006). Habituation in rodents: a review of behavior, neurobiology, and genetics. Neuroscience & Biobehavioral Reviews. *30*(7), 1045-1064.

Lever, C., Burton, S., and O'Keefe, J. (2006). Rearing on hind legs, environmental novelty, and the hippocampal formation. Reviews in the Neurosciences. *17*(1-2), 111-134.

Levin, E. D., Briggs, S. J., Christopher, N. C., and Auman, J. T. (1994). Working memory

performance and cholinergic effects in the ventral tegmental area and substantia nigra. Brain Research. *657*(1-2), 165-170.

Levine, M. S., Altemus, K. L., Cepeda, C., Cromwell, H. C., Crawford, C., Ariano, M. A., Drago, J., Sibley, D. R., and Westphal, H. (1996). Modulatory actions of dopamine on NMDA receptor-mediated responses are reduced in D1A-deficient mutant mice. Journal of Neuroscience. *16*(18), 5870-5882.

Liang, C. L., Sinton, C. M., and German, D. C. (1996). Midbrain dopaminergic neurons in the mouse: co-localization with Calbindin-D28K and calretinin. Neuroscience. *75*(2), 523-533.

Lin, J. Y. (2011). A user's guide to channelrhodopsin variants: features, limitations and future developments. Experimental Physiology. *96*(1), 19-25.

Lynd-Balta, E., and Haber, S. N. (1994). Primate striatonigral projections: a comparison of the sensorimotor-related striatum and the ventral striatum. Journal of Comparative Neurology. *345*(4), 562-578.

Matsumoto, M., and Hikosaka, O. (2009). Two types of dopamine neuron distinctly convey positive and negative motivational signals. Nature. *459*(7248), 837-841.

Markowitz, J. E., Gillis, W. F., Beron, C. C., Neufeld, S. Q., Robertson, K., Bhagat, N. D., Peterson, R. E., Peterson, E., Hyun, M., Linderman, S. W., Sabatini, B. L., and Datta, S. R. (2018). The striatum organizes 3D behavior via moment-to-moment action selection. Cell. *174*(1), 44-58.

Markowitz, J. E., Gillis, W. F., Jay, M., Wood, J., Harris, R. W., Cieszkowski, R., Scott, R., Brann, D., Koveal, D., Kula, T., Weinreb, C., Osman, M. A. M., Pinto, S. R., Uchida, N., Linderman, S. W., Sabatini, B. L., and Datta, S. R. (2023). Spontaneous behaviour is structured by reinforcement without explicit reward. Nature. *614*, 1-10.

Martinez, M. C., Zold, C. L., Coletti, M. A., Murer, M. G., and Belluscio, M. A. (2022). Dorsal striatum coding for the timely execution of action sequences. Elife. *11*. DOI: 10.7554/eLife.74929.

McHaffie, J. G., Stanford, T. R., Stein, B. E., Coizet, V., and Redgrave, P. (2005). Subcortical loops through the basal ganglia. Trends in Neurosciences. *28*(8), 401-407.

Méndez-Couz, M., Conejo, N. M., González-Pardo, H., and Arias, J. L. (2015). Functional interactions between dentate gyrus, striatum and anterior thalamic nuclei on spatial memory retrieval. Brain Research. *1605*, 59-69.

Menegas, W., Babayan, B. M., Uchida, N., and Watabe-Uchida, M. (2017). Opposite initialization to novel cues in dopamine signaling in ventral and posterior striatum in mice. Elife, *6*. DOI: 10.7554/eLife.21886.

Moaddab, M., and McDannald, M. A. (2021). Retrorubral field is a hub for diverse threat and aversive outcome signals. Current Biology. *31*(10), 2099-2110.

Morrens, J., Aydin, Ç., van Rensburg, A. J., Rabell, J. E., and Haesler, S. (2020). Cue-evoked dopamine promotes conditioned responding during learning. Neuron. *106*(1), 142-153.

Murase, S., and McKay, R. D. (2006). A specific survival response in dopamine neurons at most risk in Parkinson's disease. Journal of Neuroscience. *26*(38), 9750-9760.

Nashmi, R., and Lester, H. A. (2006). CNS localization of neuronal nicotinic receptors. Journal of Molecular Neuroscience. *30*(1-2), 181-184.

Nashmi, R., Xiao, C., Deshpande, P., McKinney, S., Grady, S. R., Whiteaker, P., Huang, Q., McClure-Begley, T., Lindstrom, J. M., Labarca, C., Collins, A. C., Marks, M. J., and Lester, H. A. (2007). Chronic nicotine cell specifically upregulates functional α4* nicotinic receptors: basis for both tolerance in midbrain and enhanced long-term potentiation in perforant path. Journal of Neuroscience. *27*(31), 8202-8218.

Neuhoff, H., Neu, A., Liss, B., and Roeper, J. (2002). Ih channels contribute to the different functional properties of identified dopaminergic subpopulations in the midbrain. Journal of Neuroscience. *22*(4), 1290-1302.

Nishino, H., Ono, T., Muramoto, K., Fukuda, M., and Sasaki, K. (1987). Neuronal activity in the ventral tegmental area (VTA) during motivated bar press feeding in the monkey. Brain Research. *413*(2), 302-313.

O'Keefe, J. (1990). A computational theory of the hippocampal cognitive map. Progress In Brain Research. *83*, 301-312.

Packard, M. G., and Knowlton, B. J. (2002). Learning and memory functions of the basal ganglia. Annual Review of Neuroscience. *25*(1), 563-593.

Paladini, C. A., Robinson, S., Morikawa, H., Williams, J. T., and Palmiter, R. D. (2003). Dopamine controls the firing pattern of dopamine neurons via a network feedback mechanism. Proceedings of the National Academy of Sciences. *100*(5), 2866-2871.

Paladini, C. A., and Roeper, J. (2014). Generating bursts (and pauses) in the dopamine midbrain neurons. Neuroscience. *282*, 109-121.

Panigrahi, B., Martin, K. A., Li, Y., Graves, A. R., Vollmer, A., Olson, L., Mensh, B. D., Karpova, A. Y., and Dudman, J. T. (2015). Dopamine is required for the neural representation and control of movement vigor. Cell. *162*(6), 1418-1430.

Parker, N. F., Cameron, C. M., Taliaferro, J. P., Lee, J., Choi, J. Y., Davidson, T. J., Daw, D. D.,

and Witten, I. B. (2016). Reward and choice encoding in terminals of midbrain dopamine neurons depends on striatal target. Nature Neuroscience. *19*(6), 845-854.

Pawlak, C. R., Ho, Y. J., and Schwarting, R. K. (2008). Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. Neuroscience & Biobehavioral Reviews. *32*(8), 1544-1568.

Paxinos, G., & Franklin, K. B. (2008). *Paxinos and Franklin's the mouse brain in stereotaxic coordinates*. Academic press.

Pidoplichko, V. I., DeBiasi, M., Williams, J. T., and Dani, J. A. (1997). Nicotine activates and desensitizes midbrain dopamine neurons. Nature. *390*(6658), 401-404.

Pierce, J. E., and Péron, J. (2020). The basal ganglia and the cerebellum in human emotion. Social Cognitive and Affective Neuroscience. *15*(5), 599-613.

Pirker, W., Djamshidian, S., Asenbaum, S., Gerschlager, W., Tribl, G., Hoffmann, M., and Brücke, T. (2002). Progression of dopaminergic degeneration in Parkinson's disease and atypical parkinsonism: a longitudinal β-CIT SPECT study. Movement Disorders. *17*(1), 45-53.

Pogorelov, V. M., Rodriguiz, R. M., Insco, M. L., Caron, M. G., and Wetsel, W. C. (2005). Novelty seeking and stereotypic activation of behavior in mice with disruption of the Dat1 gene. Neuropsychopharmacology. *30*(10), 1818-1831.

Prager, E. M., Dorman, D. B., Hobel, Z. B., Malgady, J. M., Blackwell, K. T., and Plotkin, J. L. (2020). Dopamine oppositely modulates state transitions in striosome and matrix direct pathway striatal spiny neurons. Neuron. *108*(6), 1091-1102.

Prensa, L., and Parent, A. (2001). The nigrostriatal pathway in the rat: a single-axon study of the relationship between dorsal and ventral tier nigral neurons and the striosome/matrix striatal compartments. Journal of Neuroscience. *21*(18), 7247-7260.

Przedborski, S. (2005). Pathogenesis of nigral cell death in Parkinson's disease. Parkinsonism & Related Disorders. *11*, S3-S7.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Redgrave, P., and Gurney, K. (2006). The short-latency dopamine signal: a role in discovering novel actions?. Nature Reviews Neuroscience. *7*(12), 967-975.

Redgrave, P., Gurney, K., and Reynolds, J. (2008). What is reinforced by phasic dopamine signals?. Brain Research Reviews. *58*(2), 322-339.

Reed, P., Mitchell, C., and Nokes, T. (1996). Intrinsic reinforcing properties of putatively neutral

stimuli in an instrumental two-lever discrimination task. Animal Learning & Behavior, 24(1), 38-45.

Reep, R. L., Cheatwood, J. L., and Corwin, J. V. (2003). The associative striatum: organization of cortical projections to the dorsocentral striatum in rats. Journal of Comparative Neurology. *467*(3), 271-292.

Reynolds, J. N., Hyland, B. I., and Wickens, J. R. (2001). A cellular mechanism of reward-related learning. Nature. *413*(6851), 67-70.

Rusu, S. I., and Pennartz, C. M. (2020). Learning, memory and consolidation mechanisms for behavioral control in hierarchically organized cortico-basal ganglia systems. Hippocampus. *30*(1), 73-98.

Schiemann, J., Schlaudraff, F., Klose, V., Bingmer, M., Seino, S., Magill, P. J., Zaghloul, K. A., Schneider, G., Liss, B., and Roeper, J. (2012). K-ATP channels in dopamine substantia nigra neurons control bursting and novelty-induced exploration. Nature Neuroscience. *15*(9), 1272-1280.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Reuden, C., Saalfeld, S., Schmid, B., Tinevez, J., White, D. J., Hartenstein, V., Eliceiri, K., Tomanak, P., and Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. Nature Methods. *9*(7), 676-682.

Schindler, A. G., Meabon, J. S., Pagulayan, K. F., Hendrickson, R. C., Meeker, K. D., Cline, M., Li, G., Sikkema, C., Wilkinson, C. W., Perl, D. P., Raskind, M. R., Peskind, E. R., Clark, J. J., and Cook, D. G. (2017). Blast–related disinhibition and risk seeking in mice and combat veterans: potential role for dysfunctional phasic dopamine release. Neurobiology of Disease. *106*, 23-34.

Schultz, W., Apicella, P., and Ljungberg, T. (1993). Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. Journal of Neuroscience. *13*(3), 900-913.

Schultz, W., Dayan, P., and Montague, P. R. (1997). A neural substrate of prediction and reward. Science. *275*(5306), 1593-1599.

Schultz, W. (1998). Predictive reward signal of dopamine neurons. Journal of neurophysiology. DOI: 10.1152/jn.1998.80.1.1.

Schultz, W. (2000). Multiple reward signals in the brain. Nature Reviews Neuroscience. *1*(3), 199-207.

Schultz, W. (2002). Getting formal with dopamine and reward. Neuron. *36*(2), 241-263.

Schultz, W. (2007). Behavioral dopamine signals. Trends in Neurosciences. *30*(5), 203-210.

Shen, W., Flajolet, M., Greengard, P., and Surmeier, D. J. (2008). Dichotomous dopaminergic control of striatal synaptic plasticity. Science. *321*(5890), 848-851.

Smith, Y., Bevan, M. D., Shink, E., and Bolam, J. P. (1998). Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience. *86*(2), 353-387.

Smith, K. S., and Graybiel, A. M. (2013). A dual operator view of habitual behavior reflecting cortical and striatal dynamics. Neuron. *79*(2), 361-374.

Smolinsky, A. N., Bergner, C. L., LaPorte, J. L., & Kalueff, A. V. (2009). Analysis of grooming behavior and its utility in studying animal stress, anxiety, and depression. *Mood and anxiety related phenotypes in mice: Characterization using behavioral tests*, 21-36.

Snyder, G. L., Fienberg, A. A., Huganir, R. L., and Greengard, P. (1998). A dopamine/D1 receptor/protein kinase A/dopamine-and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. Journal of Neuroscience. *18*(24), 10297-1030

Snyder, G. L., Allen, P. B., Fienberg, A. A., Valle, C. G., Huganir, R. L., Nairn, A. C., and Greengard, P. (2000). Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. Journal of Neuroscience. *20*(12), 4480-4488.

Solié, C., Girard, B., Righetti, B., Tapparel, M., and Bellone, C. (2022). VTA dopamine neuron activity encodes social interaction and promotes reinforcement learning through social prediction error. Nature neuroscience. *25*(1), 86-97.

Sonders, M. S., Zhu, S. J., Zahniser, N. R., Kavanaugh, M. P., and Amara, S. G. (1997). Multiple ionic conductances of the human dopamine transporter: the actions of dopamine and psychostimulants. Journal of Neuroscience. *17*(3), 960-974.

Spielewoy, C., Roubert, C., Hamon, M., Nosten, M., Betancur, C., and Giros, B. (2000). Behavioural disturbances associated with hyperdopaminergia in dopamine-transporter knockout mice. Behavioural Pharmacology. *11*(3-4), 279.

Stoof, J. C., and Kebabian, J. W. (1981). Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. Nature. *294*(5839), 366-368.

Stopper, C. M., Maric, T. L., Montes, D. R., Wiedman, C. R., and Floresco, S. B. (2014). Overriding phasic dopamine signals redirects action selection during risk/reward decision making. Neuron. *84*(1), 177-189.

Sturman, O., Germain, P. L., and Bohacek, J. (2018). Exploratory rearing: a context-and stress-sensitive behavior recorded in the open-field test. Stress. *21*(5), 443-452.

Surmeier, D. J., Bargas, J., Hemmings Jr, H. C., Nairn, A. C., and Greengard, P. (1995). Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. Neuron. *14*(2), 385-397.

Surmeier, D. J., Ding, J., Day, M., Wang, Z., and Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends in Neurosciences. *30*(5), 228-235.

Thorn, C. A., Atallah, H., Howe, M., & Graybiel, A. M. (2010). Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. Neuron. *66*(5), 781-795.

Thorndike, E. L. (1911). Animal intelligence: Experimental studies. The Macmillan Company, NY.

Tobler, P. N., Fiorillo, C. D., and Schultz, W. (2005). Adaptive coding of reward value by dopamine neurons. Science. *307*(5715), 1642-1645.

Tsetsenis, T., Badyna, J. K., Wilson, J. A., Zhang, X., Krizman, E. N., Subramaniyan, M., Yang, K., Thomas, S. A., and Dani, J. A. (2021). Midbrain dopaminergic innervation of the hippocampus is sufficient to modulate formation of aversive memories. Proceedings of the National Academy of Sciences. *118*(40). DOI: 10.1073/pnas.2111069118.

Tukey, D. S., and Ziff, E. B. (2013). Ca2+-permeable AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and dopamine D1 receptors regulate GluA1 trafficking in striatal neurons. Journal of Biological Chemistry. *288*(49), 35297-35306.

Ung, K., and Arenkiel, B. R. (2012). Fiber-optic implantation for chronic optogenetic stimulation of brain tissue. JoVE (Journal of Visualized Experiments). *68*. DOI: 10.3791/50004.

Walsh, R. N., and Cummins, R. A. (1976). The open-field test: a critical review. Psychological Bulletin. *83*(3), 482.

Wilson, F. A., and Goldman-Rakic, P. S. (1994). Viewing preferences of rhesus monkeys related to memory for complex pictures, colours and faces. Behavioural Brain Research. *60*(1), 79-89.

Wong, J. Y., Clifford, J. J., Massalas, J. S., Kinsella, A., Waddington, J. L., and Drago, J. (2003). Essential conservation of D 1 mutant phenotype at the level of individual topographies of behaviour in mice lacking both D 1 and D 3 dopamine receptors. Psychopharmacology. *167*, 167-173.

Xiao, C., Cho, J. R., Zhou, C., Treweek, J. B., Chan, K., McKinney, S. L., Yang, B., and Gradinaru, V. (2016). Cholinergic mesopontine signals govern locomotion and reward through dissociable midbrain pathways. Neuron. *90*(2), 333-347.

Xue, J., Qian, D., Zhang, B., Yang, J., Li, W., Bao, Y., Qiu, S., Fu, Y., Wang, S., Yuan, W., and Lu, W. (2022). Midbrain dopamine neurons arbiter OCD-like behavior. Proceedings of the National Academy of Sciences. *119*(46). DOI: 10.1073/pnas.2207545119.

Yttri, E. A., and Dudman, J. T. (2016). Opponent and bidirectional control of movement velocity in the basal ganglia. Nature. *533*(7603), 402-406.

Zénon, A., Devesse, S., and Olivier, E. (2016). Dopamine manipulation affects response vigor independently of opportunity cost. Journal of Neuroscience. *36*(37), 9516-9525.