

Exploring the functional interactions between the D2-receptors and L-type calcium channels in the control of striatal dopamine release

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Gayathri Karthik

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Christ Church, Oxford

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Abstract

Dopamine (DA) release by nigrostriatal DA neurons is critical for selection and learning of movement and motivation. Parkinson's disease (PD) is characterized by the loss of these neurons and the resultant decrease in DA. Release of DA is governed by presynaptic voltage gated calcium channels (VGCCs). The entry of calcium to these DA neurons through the L-type VGCC has been hypothesised to be a possible cause of susceptibility to degeneration. Therefore, understanding the modulation of L-type VGCCs could be important to understanding their role in PD. DA release can be strongly regulated by D2 autoreceptors (D2Rs) which act through $G\alpha_{i/o}$-PCR coupled mechanisms. They decrease transmitter release via a βγ-mediated inhibition of VGCCs. To investigate the functional interaction between D2 autoreceptors and L-type calcium channels in modulating release of dopamine, I use fast scan cyclic voltammetry to detect DA release in mouse acute striatal sections at carbon fibre microelectrodes with sub-second temporal resolution.

In the CPu, D2 receptors do not modulate L-type VGCCs; there is a lack of functional interaction between D2Rs and L-type VGCCs in controlling striatal DA release. The addition of an L-channel blocker in the presence of a D2 receptor antagonist did not reduce DA release in a manner significantly different from the addition of the L-channel blocker in the absence of the D2 antagonist. This lack of effect was maintained even in the presence of cocaine which increases extracellular DA and therefore the activation of D2 receptors. In the NAc, D2 autoreceptors inhibit the activity of the L-type calcium channels. The addition of isradipine in the presence of D2 antagonist in the NAc unmasked a role for the L-type channel which was previously shown to not participate in DA release. These experiments shed some light on the multiple layer of control of the L-type VGCCs in the NAc furthering our understanding of what factors render neurons susceptible to degeneration in PD,

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Abbreviations

AC	Adenylyl cyclase
ACh	Acetylcholine
aCSF	artificial cerebrospinal fluid
AMPARs	A-amino-3-hydroxy-5-methyl-4-isoxazolepropionoic acid receptor
ANOVA	analysis of variance
cAMP	Cyclic adenosine monophoshpate
CFM	Carbon fibre microelectrode
ChI	Cholinergic Interneuron
CPu	Caudate-Putamen (dorsal striatum)
D1/D2R	Dopamine receptors 1/2
DA	Dopamine
DAT	Dopamine uptake transporter
$[DA]_o$	Extracellular DA concentration
DHβE	dihydro-β-erythroidine
FSCV	fast scan cyclic voltammetry
GPCR	G Protein-coupled receptor
M1R	Muscarinic receptor 1
MSN	Medium spiny neuron
NA	Noradrenaline
NAc	nucleus accumbens (ventral striatum)
nAChR	nicotinic receptor
PD	Parkinson’s disease
S.E.M.	standard error of the mean
SNARE	Soluble NSF attachment protein receptor
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
SPN	Spiny projection neuron

TH	tyrosine hydroxylase
VGCC	Voltage gated calcium channels
VTA	ventral tegmental area
WT	Wild type

1.Introduction

1.1. Overview

Dopamine, a catecholamine, is a key neurotransmitter in the reward-motivation pathway (Beaulieu & Gainetdinov, 2011). Dopamine (DA) is synthesised and released in the brain by dopaminergic neurons that originate in the substantia nigra (SNc) and the ventral tegmental area (VTA). One of the most well recognized dysfunctions of the dopaminergic pathway results in Parkinson's disease. The preferential loss of dopaminergic neurons in the nigrostriatal pathway results in low dopamine in the dorsal striatum (Fearnley & Lees, 1991). One hypothesis for this preferential loss of dopaminergic neurons in the SNc as opposed to the VTA is the difference in handling of calcium by these two groups of neurons. DA neurons show autonomous pacemaking activity (Grace & Bunney, 1984a). SNc neurons rely mainly on L-type voltage gated Ca^{2+} channels to support this pacemaking while VTA neurons rely on Na^{+} channels (Guzman *et al.*, 2009; Khaliq & Bean, 2010). This calcium handling has been hypothesised to predispose nigral neurons to damage by increasing basal oxidative stress.

Release of DA is governed by presynaptic voltage gated calcium channels (VGCCs). SNc neurons project to the dorsal striatum and the VTA neurons project to the ventral striatum. L-type VGCCs have been shown to be key modulators of DA release in the dorsal striatum but not so in the ventral striatum (Brimblecombe *et al.*, 2015). Therefore, understanding the modulation of L-type VGCCs could be important to understanding their role in PD. DA release is also strongly regulated by D2 autoreceptors (D2Rs) which act through $G\alpha_{i/o}$-PCR coupled mechanisms. They decrease transmitter release via several mechanisms including a βγ-mediated inhibition of VGCCs.

In this work, I will explore the interactions between the D2Rs and the L-type VGCCs in modulating DA release.

1.2. Basal Ganglia and the striatum

The main source of dopamine (DA) in the central nervous system is from dopaminergic neurons in the midbrain (Chinta & Andersen, 2005). The cell bodies of dopaminergic neurons arise from the substantia nigra (SNc) and the ventral tegmental area (VTA) and play a key role in basal ganglia function. The basal ganglia are a group of subcortical structures in the human forebrain that are associated with various functions including cognition, voluntary movements and procedural learning. The basal ganglia have been hypothesised to play a central role in action selection and learning reinforcement (Stocco *et al.*, 2010). It has also been implicated in several neuropsychiatric diseases as well as devastating neuromotor conditions such as Parkinson's disease and Huntington's disease (Ring & Serra-Mestres, 2002). The basal ganglia receive inputs from the neocortex and projects to the thalamic nuclei, thereby influencing executive functions as well as cognitive and motor functions (Figure 1.1)(Purves et al., 2001).

1.2.1. Basal Ganglia anatomy and physiology

The basal ganglia comprise of four main subcortical nuclei; the striatum, the substantia nigra, the Globus pallidus and the subthalamic nucleus. Most of the neurons in the basal ganglia are projection neurons and GABAergic (Tepper *et al.*, 2007). The anatomical organization of the basal ganglia is most commonly described using the rate model theory (DeLong, 1990) (Figure 1.1) The rate model theory is based on clinical observations of brain lesions. It describes two parallel circuits modulated by the midbrain. The direct pathway is motor permissive (Go) while the indirect pathway is motor-suppressive (No-go) (Purves et al., 2001).

The direct pathway is formed by projections from the medium spiny neurons (MSN) of the dorsal striatum, composed of the caudate and the putamen, to the internal circuit of the globus pallidus and the substantia nigra pars reticulata (SNpr) (Purves et al., 2001). This pathway facilitates the initiation and execution of voluntary movements. At rest, the SNpr sends spontaneous signals to the thalamus which in turn projects to the motor cortices to relieve tonic motor neurons from tonic inhibition (Purves *et al.,* 2001). The indirect pathway consists of projections to the external segment of the globus pallidus and the subthalamic nucleus. This pathway serves to modulate the disinhibitory actions of the direct pathway (Figure 1.1). The direct pathway is made up of MSNs which mainly express D1- subtype dopamine receptors, while MSNs in the indirect pathway selectively express more D2-type receptors. A small number of spiny neurons express both D1 and D2Rs (Gagnon et al., 2017). MSNs constitute up to 95% of total striatal neurons (Kemp & Powell, 1971). They receive inputs from various areas of the brain with the majority of innervation coming from the prefrontal cortex and thalamus (C.R. Gerfen & Bolam, 2017).

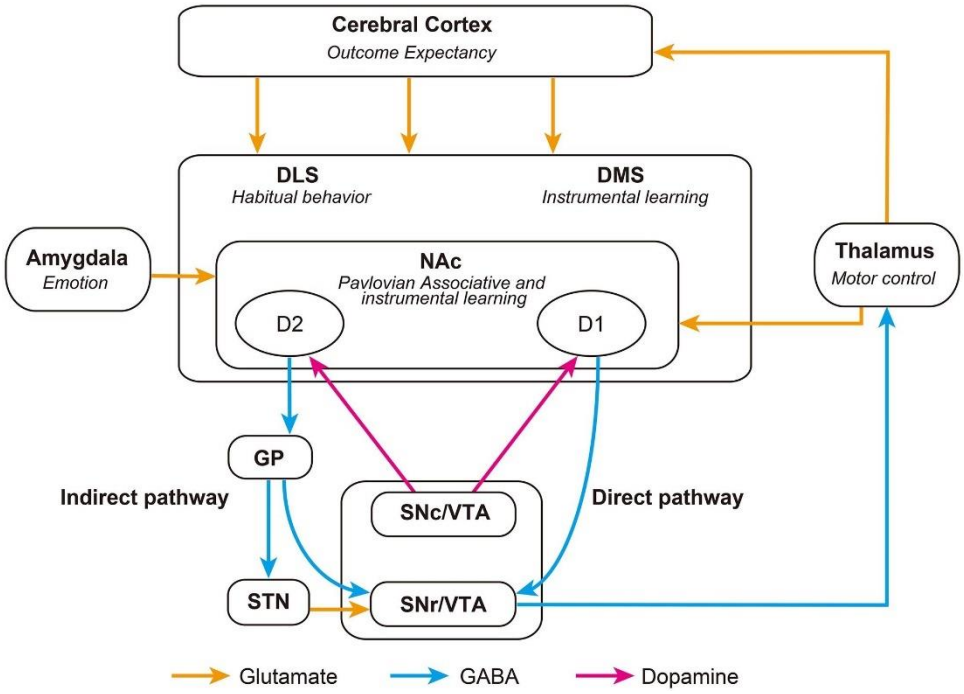


Figure 1.1. Direct and indirect pathways in the basal ganglia. Nigrostriatal D1 *direct* pathway neurons inhibit the SNr and release inhibition of thalamic activity, promoting behaviour. Nigrostriatal D2 *indirect* pathway neurons inhibit the GP, disinhibiting the STN and exciting the SNr, which ultimately inhibits the thalamus and thus suppresses behaviour. The balance between these opposing projections is likely to be regulated by glutamatergic and dopaminergic afferents, as well as GABAergic signaling within the striatum. DLS, Dorsolateral Striatum; DMS, Dorsomedial Striatum; GP, Globus Pallidus; NAc, Nucleus Accumbens; SNc, Substantia Nigra pars compacta; SNr, Substantia Nigra pars reticulata; STN, Subthalamic Nucleus; VTA, Ventral Tegmental Area. Adapted from Macpherson, Morita, & Hikida, 2014.

Dopaminergic inputs to the striatum arise from the midbrain. The mesostriatal dopaminergic system comprises of the mesolimbic and nigrostriatal pathways that include dopaminergic projections from the ventral tegmental area (VTA) and the substantia nigra (SNc) to the striatum respectively. These pathways play a major role in processing limbic and motor function (Molochnikov & Cohen, 2014).

1.2.2. DA neurons physiology

DA neurons exhibit two main firing properties; regular tonic firing and brief high frequency bursts. In the absence of excitatory input, DA neurons have the ability to fire spontaneously at slow rates (Grace & Bunney, 1984a, 1984b). Tonic spiking in DA neurons is low-frequency, 2-10 Hz and the spiking is rhythmic and can occur spontaneously *in vitro.*

The autonomous pace making is driven by a subthreshold oscillation called slow oscillatory potential (SOP) which persist after pharmacological blockade of spiking-generating currents (Ping & Shepard, 1999). The oscillations can be recorded at the soma and as well as dendrites indicating that the mechanism is in place through the dendrites (Wilson & Callaway, 2000). The tonic activity is responsible for the basal concentration of DA and has been shown to induce a steady state $[DA]_o$ (B Jill Venton et al., 2003).

The SOPs are caused by oscillations in intercellular Ca^{2+} concentrations caused by the opening of Ca_v1 channels. DA neurons in the SNc and VTA have been shown to have vastly different reliance on Ca^{2+} for generation of the SOPs. DA neurons in the SNc depend on Ca^{2+} for this pace-making activity, while DA neurons in the VTA depend on Na^+ (Khaliq & Bean, 2010). Juvenile mice DA neurons in the SNc and VTA use Na^+ currents to drive their SOPs but upon reaching adulthood, SNc

neurons switch to a Ca^{2+} driven current while VTA neurons remain using Na^{+} (Chan et al., 2007). Phillipart *et al.* (2016) have shown that indeed, the amplitude and contribution of the L-type Ca^{2+} current is higher in the SNc while the VTA appears to depend more on the N-type channel for contribution to somatic calcium currents.

The pathological identity of Parkinson's Disease is the loss of neurons in the SNc and the degeneration of SNc projections to the CPu in the striatum ((Kordower et al., 2013). Neurons in the VTA appear less susceptible to such degeneration. The susceptibility of SNc neurons has been linked to several factors. The first is the Ca^{2+} driven pacemaking and the resultant high metabolic demand causing high oxidative stress in these neurons (Wallace, 2005). An inverse correlation has been also shown between the risk of PD and the expression of intercellular calcium buffering proteins such as calbindin suggesting that the loss of localized control of intercellular Ca^{2+} may increase vulnerability (Surmeier et al., 2017; Yamada *et al.*, 1990). Ca^{2+} is also known to modulate expressions of enzymes involved in DA production as well as promote the entry of Ca^{2+} into the mitochondria and the stimulation of oxidative phosphorylation. This results in the production of reactive oxygen species and reactive nitrogen species that in turn damage proteins, lipids and DNA causing vulnerability of these neurons.

Dopaminergic neurons in the VTA play central roles in goal-directed as well as reward seeking behaviour (Morales & Margolis, 2017). Neurons in the VTA are reciprocally connected with many areas in the brain with neurons projecting to the prefrontal cortex and striatum. The efferent fibres from VTA neurons give rise to two main pathways; the meso-cortical and meso-limbic pathways which are involved with emotion-based behaviour such as reward and motivation. While only 1% of all neurons are dopaminergic neurons, they innervate areas involved with motor

behaviour, motivation and working memory. This is especially true of reward-based learning-behaviours (Schultz, 2000). The nigrostriatal pathway is key in voluntary motor movement. Nigral neurons play a key role in eye movement, motor planning, reward seeking, learning and addiction. Most of this behaviour is mediated by the striatum (Nicola *et al.*, 2000).

1.2.3. Striatum

The striatum itself is the main input nuclei in the basal ganglia. In humans, the dorsal striatum consists of the caudate and the putamen while the ventral striatum consists of the nucleus accumbens and the olfactory tubercle (*Rice et al.,* 2011). It acts as a key modulator of different aspects of cognition, motor, action planning and decision making. The striatum is largely a GABAergic structure comprised mainly of medium spiny neurons (MSNs) and the interneurons. It receives dopaminergic and glutamatergic afferents from the cortex and other areas of the basal ganglia as well as from limbic structures such as the amygdala, hippocampus, thalamus, entorhinal cortex and inferior temporal gyrus (Purves et al., 2001)

The projection neurons or medium spiny neurons make up 90-95% of all striatal neurons. MSNs can be further subdivided into those that express D1Rs and those that express D2Rs. Striatal interneurons make up between 5-10% of all striatal neurons and can be characterized into four main types: cholinergenic interneurons (ChIs), fast-spiking interneurons (FSIs), calretenin-expressing interneurons, and persistent and low threshold spiking interneurons (PLTSIs). Interneurons play key roles in striatal functions, GABAergic inhibition and modulation of the spiny neurons (Silberberg & Bolam, 2015).

The dorsal striatum comprising of the caudate and the putamen (CPu) receives dopaminergic inputs mainly from the SNc and glutamatergic afferent from the motor cortex. It is associated with sensorimotor function and mediates cognition and stimulus-response learning. The main source of dopaminergic neurons for the ventral striatum comprising of the nucleus accumbens core and shell (NAc) is from the VTA. The ventral striatum receives cortical inputs from the prefrontal cortex. The ventral striatum mediates reward cognition, motivation and reinforcement (Haber *et al.*, 2000).

Given that the CPu and the NAc are functionally different, driving motor control and limbic behaviours respectively, they are also physiologically different in various aspects. Studies *in vivo* and *in vitro* have shown a gradient from the dorsal to the ventral striatum in the absolute concentration of DA release as well as in reuptake kinetics (Cragg *et al.*, 2002). DA release and reuptake occur faster in the CPu than in the NAc. Factors that control extracellular concentration of DA will determine the neuromodulatory effects of DA. Two such mechanism central to the transmission of DA in the striatum is the inhibition of release of DA via pre-synaptic D2 autoreceptors (D2Rs) and the re-uptake of DA via the Dopamine transporter (Cass & Gerhardt, 1994; Trout & Kruk, 1992).

The activation and time course of activity of D2Rs have been shown to be vastly different between the two regions. The time window in which D2Rs are active in the NAc is longer than that of D2Rs in the CPu. D2Rs in the both the CPu and NAc begin to activate 200 ms after initial DA release. However, the peak dopamine autoreceptor activation occurs earlier in the CPu at ~ 550ms while it occurs later at ~ 700ms in the NAc (Phillips *et al.*, 2002). Anzalone *et al.,* (2002) found that D2 receptors on MSNs can have an inhibitory effect on the release of DA. Marcott *et al.* (2018) also found that the sensitivity of D2 receptors on MSNs to DA in the striatum is higher in the NAc compared to the CPu. They also found that these D2 receptors mediated synaptic currents were smaller in the NAc compared to the CPu.

The termination of DA signals has been shown to occur faster in the CPu than the NAc with greater uptake rates in the dorsal striatum. This is attributed to the higher expression and activity of the DAT in the CPu (Calipari et al., 2012). DAT expression in the striatum follows a gradient from the dorso-lateral CPu to the NAc (Cragg *et al.,* 2002).

1.3. Factors mediating striatal transmission of dopamine

DA neurotransmission is mediated and regulated at many levels including synthesis, uptake, vesicular transport, and Ca^{2+} homeostasis (Sulzer, Cragg, & Rice, 2016)

1.3.1. Acetylcholine receptor modulation of striatal DA transmission

The striatum is a heterogenous mix of many cell types and is very GABAergic structure. Cholinergic interneurons (ChIs) constitute 1% of the types of neurons in the striatum (Kawaguchi *et al.*, 1997). These interneurons are large and aspiny and were first described in the late 1800s. ChIs are thought to be the source of striatal acetylcholine (ACh) (Woolf, Butcher, & BUTCHER Cholinergic, 1981).

ACh plays a dominant role in shaping the release and short-term plasticity. Nicotinic ACh receptors (nAChRs) are expressed throughout the striatum. They act as extremely powerful modulators of striatal DA release, despite making up only 1% of the striatal cells, they project extensively and have been estimated to produce 500, 000 varicosities (Bolam *et al.*, 1984). The highest levels of cholinergic markers are found in the striatum (Hebb & Silver, 1956).

The axonal terminals of DA neurons in the striatum have been shown to express nAChRs and are modulated by the release of ACh from ChIs. DA evoked by single-pulse and low frequency pulse train stimulations are suppressed when nAChRs are antagonised. However, this antagonism also relieves the short-term depression that limits a subsequent DA release (Rice & Cragg, 2004). They are also tonically active and synchronous activation of ChIs with nAChRs on DA axons can drive release of DA independently of DA neuron activity (Cachope et al., 2012; Threlfell et al., 2012).

1.3.2. Calcium channels in neurotransmitter release

Neurotransmitter release from vesicles requires the influx of Ca^{2+} via voltage gated calcium channels (VGCCs) which open upon depolarization (Katz & Miledi, 1968). The opening of these channels allows entry of calcium ions into the cytosol, increasing intercellular calcium levels, which is usually maintained at nM levels, to trigger a range of processes including neurotransmitter release, gene transcription, neurite growth as well as the activation of calcium dependent enzymes like CaMKII and PKC (Clapham, 2007).

VGCCs are broadly classified into high voltage activated and low voltage activated. The pore forming subunit (α1) which gives the channel its pharmacological properties is also used to classify the channels; there are 10 known genes for the α1 subunit (Simms & Zamponi, 2014). The α1 subunit is the largest and incorporates the conduction pore, the voltage sensor and the gating apparatus. It forms the channel along with ancillary subunits βγ and α2δ which modulate the trafficking of the channels (Dolphin, 2013; Takahashi *et al.*, 1987).

The genetic identity of the α1 subunit is used to classify the channels. There are three main types of the α1 subunit; Ca_v1, Ca_v2 and Ca_v3. Subtypes in each of these categories arise from splice variants of these genes. Ca_v1 encodes for L-type channels. $Ca_v2.1$ gives rise to P-/Q-type channels, $Ca_v2.2$ to N-type channels and $Ca_v2.3$ encodes for R-type channels (Bourinet et al., 1999; Soong et al., 1993). Ca_v3 gives rise to T-type channels (Perez-Reyes et al., 1998). The properties of these channels and the ligands used to study their pharmacological properties are outlined in Table 1.

Type of channel	Gene Name	Localization	Characteristics	Ligands & Pharmacological agents
L-type calcium channel	$Ca_v1.1$	Skeletal Muscle	Slow voltage dependent gating	Dihydropyridines
	$Ca_v1.2$	Neuronal/Cardiac		
	$Ca_v1.3$	Neuronal/Cardiac		
	$Ca_v1.4$	Retina		
P-/Q-type channel	$Ca_v2.1$	Neuronal		ω- agatoxin IVA (P at low concentration and Q at high concentration)
N- type	$Ca_v2.2$	Neuronal		ω- agatoxin GIVA and MVIIA
R-type	$Ca_v2.3$	Neuronal		SNX-482 and Nickel at mM concentrations
T-type calcium channels	$Ca_v3.1$	Neuronal	Lack ancillary subunits (β and α2δ)	Mibefridil and derivates
	$Ca_v3.2$	Neuronal		
	$Ca_v3.3$	Neuronal		

Table 1. Classification and types of voltage gated calcium channels (adapted from Simms & Zamponi, 2014)

Classically most synapses are thought to rely on N-type and P/Q-type channels for neurotransmitter release (Wheeler *et al.,*1994), although all calcium channels appear to support neurotransmission, with the Ca_V2 modulating fast synaptic transmission, Ca_V1 driving slower, tonic release and Ca_V3 driving low-threshold exocytosis (Simms & Zimponi, 2014).

1.3.2.1 Vesicle release machinery

The vesicle release machinery governing the release of DA has several proteins that play a central role in membrane fusion and release. In brief, the mechanics of membrane fusion involves the bringing together of the vesicle and cell membrane in, the destabilization caused by the bending of the bilayers, the fusion of the bilayer leaflets forming a stalk intermediate, the merger of the leaflets to form a fusion pore and finally the expansion of this pore (Rizo & Xu, 2015) (Figure 1.2).

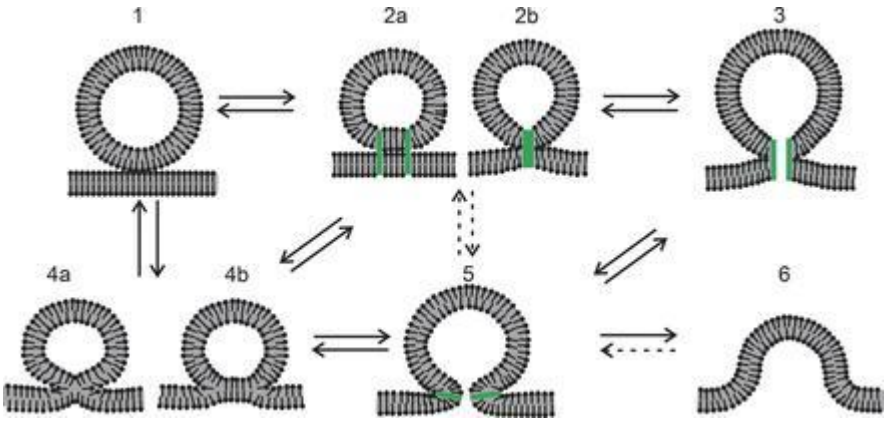


Figure 1.2. Protein elements are coloured green. (1) A vesicle approaches the plasma membrane. (2) Proteins hold the vesicle and plasma membrane together, either through separate contacts (a) or through one central contact (b). (3) A proteinaceous fusion pore could form from a central contact as in 2b. (4) Lipid mixing of the outer (proximal) leaflets can begin, first through the formation of a stalk (4a) and then through the formation of an extended hemifusion diaphragm (4b) in which the fused proximal leaflets retract and leave a bilayer formed by the two distal leaflets. (5) A fusion pore formed by a contiguous lipid bilayer curved into an hourglass-like shape. (6) A greatly expanded lipid fusion pore on the way to complete merger of the plasma and vesicle membranes. Adapted from Chang, Chiang, & Jackson, 2017.

SNARE proteins are vital for the trafficking of intercellular membranes. Neuronal SNAREs that mediate exocytosis are the synaptic vesicle protein Synaptobrevin, the plasma membrane protein SNAP-25 and syntaxin-1. The tight assembly of these three proteins creates a trans-SNARE complex which bring the two membranes into

proximity. The energy released by the formation of this assembly is used to induce membrane fusion. Upon fusion a cis-SNARE complex is formed, although the mechanism for this change in conformation is not yet thoroughly understood (Südhof, 2012) (Figure 1.3).

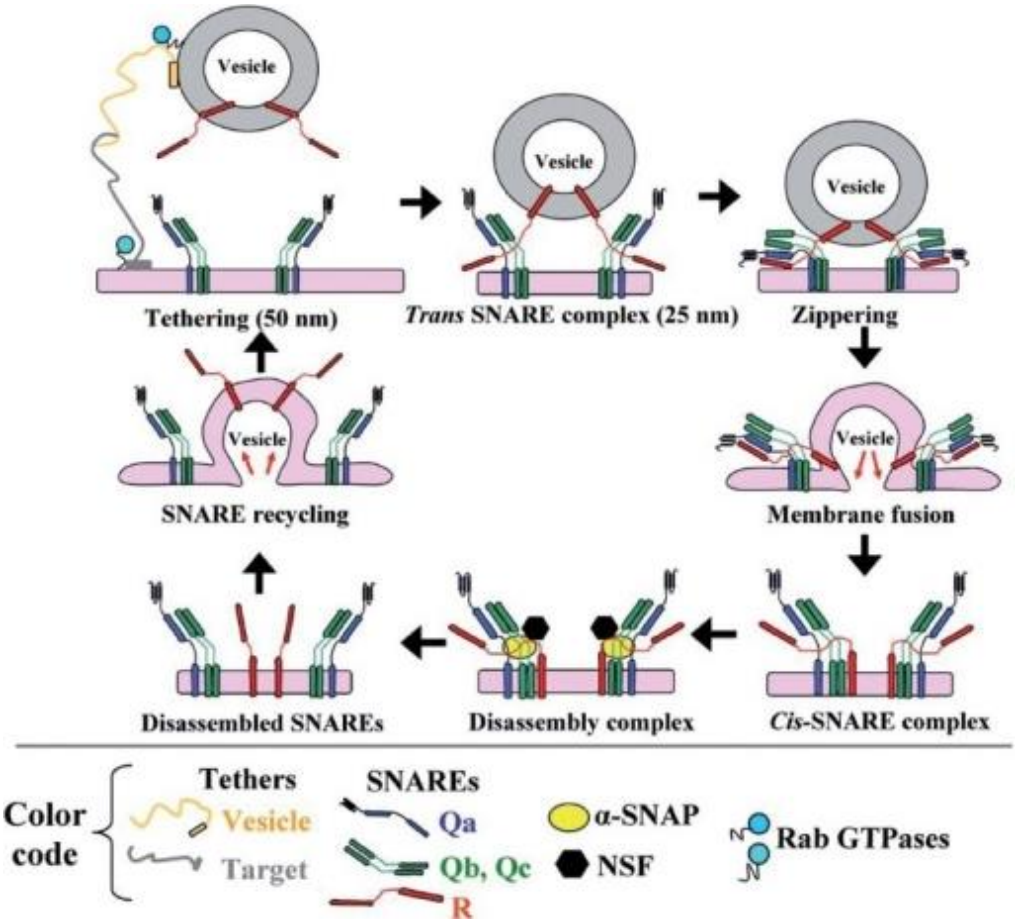


Figure 1.3. SNARE protein complex: The SNARE cycle in membrane fusion. Initial interaction between a vesicle and its target membrane is mediated by cognate tethers. Membrane fusion is then mediated by the formation of a quaternary-α-helical trans-SNARE complex consisting of one v- (or R -) SNARE on the vesicle and two or three t- (or Q-) SNAREs on the target membrane. After membrane fusion, the cis-SNARE complex is disassembled by the ATPase NSF which is recruited to the cis-SNARE complex from the cytosol by α-SNAP. (Adapted from Sehgal & Lee, 2011)

In most synapses, P/Q and N-type VGCCs were thought to drive neurotransmitter release by providing ways for calcium channel entry (T. Takahashi & Momiyama, 1993). Additionally, in these subtypes, the presence of a synaptic protein interaction site (synprint) between transmembrane domains II and III, allows for the direct interaction with the SNARE proteins (Sheng, Rettig, Cook, & Catterall, 1996; Simms & Zamponi, 2014). The synprint interacts with SNARE proteins in the vesicle release complex, allowing for exocytosis of the vesicles. R-type channels has also been shown to localise presynaptically and participate in the release of neurotransmitters however they lack the synprint site and associate with the SNARE complex functionally (Kemp & Powell, 1971). T-type calcium channels, like R-type channels lack the synprint site but can also associate with SNARE proteins (Weiss et al., 2012). L-type channels do not have a synprint site and are therefore mostly found at post synaptic sites and are anchored by SHANK proteins (H. Zhang et al., 2005). However, $Ca_v1.3$ have been found pre-synaptically in cochlear hair cells (Striessnig, 2013).

1.3.2.2. VGCCs in striatal DA release

Release of DA in the striatum is catalysed by pre-synaptic VGCCs in the axons of dopaminergic neurons from the SNc and the VTA. In the CPu, N-, P/Q- channels have been shown to modulate DA release (Phillips et al., 2002).

Brimblecombe *et al.* (2015) identified the VGCCs involved in DA release in the CPu and the NAc without the confounding effect of cholinergic interneurons and showed that significant differences in the types of channels involved. In the CPu, Brimblecombe *et al.* (2015) found, using fast scan cyclic voltammetry, that the release of DA is governed by N- and Q- channels. Additionally, they also found that L-type and T-type VGCCs, which are not usually associated with neurotransmitter release, also influenced DA release in the CPu. This influence of L- type and T-type however were absent in the NAc where they found that DA release was mainly driven by N- and Q-type channels. However, in the NAc, it was found that L-type and T-type channel involvement could be unmasked by elevating extracellular calcium.

1.3.3. D2 autoreceptors and striatal DA transmission

The physiological effects of DA are modulated via five sub-types of the dopamine receptors which all belong to the large G-protein coupled metabotropic receptor family (GPCRs). Activation of these receptors is mediated via the interaction of the GTP-binding proteins. The DA receptors act by activating or inhibiting the cAMP pathway. Mammalian DA receptor subtypes fall into two major groups: D1-like and D2-like receptors. Members of the D1 family (D1 & D5) are $G\alpha_{s/olf}$ coupled GPCRs and activate adenylyl cyclase and increase cAMP levels leading to the excitability of cells (D James Surmeier et al., 2010). D2-like receptors (D2, D3 and D4) are inhibitory and couple to $G\alpha_{i/o}$ to inhibit cAMP production. They also reduce

excitability of the cell by activating inwardly rectifying potassium channels (GIRK) (Neve *et al.*, 2004).

Located presynaptically, autoreceptors act as negative feedback mechanisms that modulate the firing rate of neurons, neurotransmitter release as well as synthesis in response to changes in neurotransmitter levels in the extracellular space. D2 receptors are key regulators of dopamine dependent transmission. D2 autoreceptors (D2Rs) reduce DA release upon activation. They modulate this change in DA release via several mechanisms (Ford, 2014). D2 auto-receptors are located on the soma and dendrites of the dopaminergic neurons in the VTA and the SNc as well as on axonal terminals in projection areas (Missale et al., 1985).

D2 receptors can be found as two main splice variants; D2L and D2S which are differentially distributed. While D2S is found predominantly presynaptically, D2L is found predominantly post-synaptically (Ford, 2014). D2S has been reported to have a higher affinity for DA and has been shown to inhibit adenylyl cyclase more effectively (Castro & Strange, 1993; Montmayeur et al., 1993). However subsequent downstream cascades have made it difficult to assign signalling roles for the variants (Romanelli et al., 2010). Studies have suggested that D2L serves as the main heteroreceptor while both forms may be autoreceptors.

1.3.3.1. D2 autoreceptor modulation of DA release

D2 receptors are inhibitory and act by inhibiting adenylyl cyclase and calcium channels. Pre-synaptic auto-receptors modulate dopamine release through three main mechanisms; through the modulation of probability of vesicular dopamine release, changing dopamine synthesis and altering DA re-uptake kinetics (Ford, 2014). The activation of the D2 receptors by dopamine decreases the subsequent probability of release. The time scale of this inhibition varies between *in vivo* and *in vitro* but is thought to range from several milliseconds to several seconds.

The simplest model for autoreceptor inhibition of DA release is via D2R dependent activation of the K^+ channel which inhibits DA release by hyperpolarising the membrane (Lacey, Mercuri, & North, 1987). In the soma this has been shown to occur through the GIRK channels while in the striatum this is through the action of $K_v1.2$ (Beckstead & Williams, 2007; Fulton et al., 2011).

D2 autoreceptors also mediate DA release through the inhibition of the tyrosine hydroxylase through cAMP-PKA. This downregulation of TH through phosphorylation of its regulatory domain results in a reduction in filling of vesicles (Pothos et al., 1998). It also alters the expression of the VMAT which fills the vesicles(Truong et al., 2004).

D2 autoreceptors also play a key role in the regulation of reuptake of the dopamine after release. Re-uptake is mediated by the DAT and the clearance of dopamine is determined by the kinetics and reuptake by the dopamine transporter (DAT). An increase of the DAT expression on the cell surface occurs after activation of the D2 autoreceptors. However, there is evidence to suggest that this occurs only when saturating amounts of dopamine is applied i.e. the tonic background firing of

dopamine neurons does not raise concentration to levels high enough to activate D2 mediated DAT regulation (Ford, 2014).

Patch clamp experiments in dissociated midbrain dopaminergic neurons have shown that D2 activation decreases Ca^{2+} currents indicating a regulation of VGCCs by the D2R. The main VGCCs through which the decrease was seen were the N- and P-type VGCCs (Cardozo & Bean, 1995).

1.3.3.2. D2 autoreceptor modulation of calcium channels

The most common mechanism through which D2R modulation of VGCCs is thought to take place is via a βγ-mediated inhibition (Herlitze et al., 1996). D2 autoreceptors have been shown to inhibit the P/Q and N-type channels (Cardazo & Bean, 1995). The interaction between D2Rs and calcium signalling is mediated by the Gβγ subunit of the G-protein which separates from the Gα subunit upon receptor activation. Inhibition occurs due to a positive shift in voltage dependence and a slowing of channel activation (Dolphin, 2013).

There is also precedence for the modulation of VGCCs that do not usually participate in neurotransmitter release by D2 receptors. In MSNs the activation of Gβγ regulated by D2 receptors increases the intercellular calcium via a PLC-IP_3 signalling cascade. This mechanism was shown to inhibit activity by L-type calcium channels (Hernandez-Lopez et al., 2000). In striatal interneurons, D2 receptors have been shown to reduce N-type calcium channel current through Gβγ signalling but in a PLC independent manner indicating that the GPCR pathway need to necessarily involve cAMP.

1.3.4. Striatal DA transmission and reuptake by the dopamine reuptake transporter

Dopamine reuptake via the DAT is the primary mechanism through which DA is cleared from the synapse. The availability of DA after release is dependent on the activity of the dopamine reuptake transporter (DAT). The DAT drives the reuptake of DA from the extracellular space thereby effectively modulating the spatio-temporal dynamics of DA transmission. As DA transmission is volume transmission, the DAT plays a key role in not just ensuring the removal of DA from synapses but also regulating the action of DA on extrasynaptic receptors (Rice et al., 2011). Belonging to the SLC6 family of transporters, the DAT transports DA to the intercellular membrane via symport. It couples the translocation of DA to movement of Na^+ and Cl^- into the cell (Pramod et al., 2013). The activity of the DAT is dependent on kinetics with which the conformational change that allows the translocation occurs. The transport capacity of the DAT is also a function of surface density.

DAT inhibitors such as cocaine and amphetamines not only decrease the rate of reuptake of extracellular DA, they are also thought to increase DA release via modulation of reserve pool of DA vesicles (Ewing et al., 1983; Venton et al., 2006).

1.3.4.1. Modulation of DAT activity

Experimental evidence has shown that there are two main ways in which the acute control of DAT activity occurs. The first is via the direct modification of functional parameters of a single protein, such as its efficacy of permeation. The second method of acute control is through the trafficking and redistribution of the DAT between the plasma membrane and endosomal compartments.

Protein phosphorylation is the most common strategy used by cells to regulate protein function. The DAT has several consensus sites for phosphorylation on the

cytoplasm-side, many of which lie on the N-terminus. The phosphorylation of a protein has profound effects on the intrinsic activity as well as the distribution of the protein and this is true for the DAT. Several kinases and phosphatases are involved in the phosphorylation of the DAT. PKC and ERK are two DAT regulation systems that have been well characterised (Ramamoorthy et al., 2011). Activation of PKC rapidly down-regulates the DA transport via DAT endocytosis (Melikian & Buckley, 1999).

The activation of GPCRs expressed on dopaminergic neurons can regulate DAT activity. The D2Rs are localised presynaptically and regulate DA release by regulating the activity of tyrosine hydroxylase as well vesicular DA release. Activation of D2Rs have been shown to acutely increase DAT expression by inserting more DAT into the cell membrane. D2 activation has also been shown to increase the V_{max} of the DAT. Studies have suggested that upon activation of D2Rs, there is upregulation of rapid trafficking of DAT to the plasma membrane. Lee *et al.* (2007), found that DAT and the D2 receptors are often co-localised on the cell surface.

1.3.4.2. DAT interaction with L-type calcium channels

The DAT is electrogenic and the uptake of DA generates an inward current. The transport of DA is coupled to two Na^+ and one Cl^- and this movement of these ions causes a depolarization of the membrane. In *Xenopus* oocytes, three conductances were associated with the activation of the human DAT (hDAT); a coupled transport associated conductance, an uncoupled transport associated conductance and a cation leak current which is constitutive (Sonders & Amara, 1996). Ingram *et al.,* (2002) suggest that DAT currents modulate the excitability of DA neurons as well as regulate release of DA. They highlighted the presence of a Cl^- conductance that can alter the excitability and firing of DA neurons (Ingram et al, 2002).

There has been recent evidence to suggest that DAT-mediated VGCC conductance can modulate DA release. L-type calcium channels of the $Ca_V1.3$ subtype have been shown to activate at more hyperpolarised potentials. Cameron *et al.* (2015) showed that the depolarization caused by the activity of the DAT was sufficient to activate L-type VGCCs.

1.4. Parkinson's disease

Parkinson's disease (PD) is one of the most common neurodegenerative diseases. It is a progressive neurodegenerative disorder characterized by the loss of motor control and co-ordination (Mazzoni et al., 2012). It refers to a condition that affects several regions of the brain including the midbrain and cortex, as well as the brainstem (Galvan & Wichmann, 2008). The symptoms of PD manifest as motor related dysfunction such as resting tremor, stiffness and slowness of movement, and are attributed to the loss of nigral dopaminergic neurons (Damier et al., 1999; Fahn, 2003).

The cause of PD in unknown, although one in ten cases can be traced to genetics. The pathology of PD appears to be regionally selective; an unknown cause of increased vulnerability of dopaminergic neurons in the substantia nigra appears to make them more susceptible to degeneration. Several hypotheses have been put forward as the potential cause of this vulnerability; the reliance on DA as a neurotransmitter, the high energy demand caused by extensive arborization or the higher mitochondrial stress caused by a Ca^{2+} driven pacemaking activity (D. James Surmeier et al., 2017).

1.4.1. Pathophysiology

The pathological identity of PD is the loss of neurons in the SNc and degeneration of SNc projections to the CPu in the striatum. The motor symptoms therefore appear before the cognitive decline. A substantial degeneration of at least 70% of nigrostriatal neurons has to occur before symptoms manifest indicating the presence of compensatory mechanisms (Bernheimer et al., 1973).

The other hallmark of PD is the accumulation of inclusion bodies called Lewy bodies made of the protein α-synuclein (Dickson et al., 2009). The accumulation of Lewy bodies results in the loss of neurons most marked in the SNc (Polymeropoulos et al., 1997). Synuclein accumulation is thought to contribute to PD in a number of ways, with the most well described being the formation of insoluble oligomeric fibrils which are neurotoxic and result in neuronal death by disrupting cell homeostasis. However, the presence of Lewy bodies and its relationship to cell death and clinical dysfunction is still not clearly defined (Schapira, 2006).

The loss of dopamine causes several secondary changes. A significant loss of dendritic spine density on medium spiny neurons in the striatum has been observed

(Zaja-Milatovic et al., 2005). This has been hypothesised to alter transmission in the cortico-striatal pathway. MSNs in the indirect pathway have also been shown to be more susceptible to loss of dendritic spines (Day et al., 2006). In the striatum, loss of dopamine has also been shown to affect the sensitivity of receptors. A quantification of D2 autoreceptor from post mortem samples of Parkinsonian brains showed elevated mRNA levels (Dragicevic et al., 2014). Grefen *et al.* (1990) found that D1 receptor mRNA were downregulated and D2 receptor mRNA was upregulated in their respective MSNs.

In the midbrain, loss of dopamine results in changes to firing pattern of neurons. This firing rate change in the basal ganglia has been explained by the 'rate model' to be caused by the disturbances in balance caused by an imbalance in activity in the direct and indirect pathway. The loss of D2 receptor activation causes a decrease in inhibition. The decrease in DA release also results in a decrease of D1 activation.

1.4.2. Pathogenesis

Several processes have been implicated in the pathogenesis of PD. These mechanisms include dysfunction of mitochondria, oxidative stress, and activation of microglia, impairment of proteasome, impaired autophagy and formation of α-synuclein aggregation. None of these mechanisms occur in isolation and there is significant overlap. The increased susceptibility of SNc neurons has been linked to the pacemaking related calcium influx, the resulting high metabolic demand causing the generation of highly reactive oxygen species causing oxidative stress in the neurons.

This high oxidative stress results in damage to the mitochondria as well as other organelles. It has also been shown to increase the aggregation of α-synuclein

thereby increasing the demand on degradative systems like the proteasome and lysosomes. The oxidation of DA undergoes further generates oxidative species. Impaired autophagy and dysfunction of the lysosome are important causes of PD pathogenesis. Aggregation of α-synuclein is cleared by proteasomes and the high level of aggregate production as well as damaged mitochondria can lead to overwhelming of these organelles.

Given the complex interactions between the complex factors that modulate DA transmission as well as the underlying calcium driven vulnerability of these neurons, understanding how all these factors modulate each other will become important in understanding the causes of PD.

1.5. Aims of this study

Dopamine (DA) release by nigrostriatal DA neurons is critical for selection and learning of movement and motivation. Parkinson's disease (PD) is characterized by the loss of these neurons and the resultant decrease in DA. Release of DA is governed by presynaptic voltage gated calcium channels (VGCCs). The entry of calcium to these DA neurons through the L-type VGCC has been hypothesised to be a possible cause of susceptibility to degeneration. Therefore, understanding the modulation of L-type VGCCs could be important to understanding their role in PD.

In the CPu, L-type VGCCs contribute to DA release. The addition of isradipine, an L-type specific calcium channel blocker of the dihydropyridine class decreased evoked DA release to 70% in the CPu (Brimblecombe *et al.,* 2015). This effect of isradipine was found to be occluded in the CPu in the presence of cocaine (Brimblecombe, unpublished data Cragg lab).

Given that there has been evidence to suggest that L-type VGCCs interact with and are modulated by both the Dopamine Transporter (DAT) (Cameron et al., 2015) and by D2 auto-receptors which have been shown to inhibit VGCCs through βγ-mediated inhibition (Cardozo & Bean, 1995), we hypothesised that the lack of the isradipine effect in the presence of cocaine is due to increased D2 receptor activation and subsequent inhibition of L-type VGCCs. This thesis investigates this modulation of L-type VGCCs by the D2 receptors in the striatum. Understanding the interactions between the D2 receptors and the L-type calcium channel in controlling striatal dopamine release will be important in understanding its role in PD.

2. Materials & Methods

2.1. Animal model for experimentation

All experiments in this work were undertaken using the mouse strain C57BL6/J. It is the most commonly used mouse strain in biomedical research (Bryant, 2011). It is a popular choice as it is physically active, capable of learning a variety of tasks and, breeds frequently allowing for robustness in experimental setup (Bryant,2011).

Animal experiments remain essential in understanding the fundamental mechanisms of many human diseases. The mouse has been used as a mammalian model organism as it offers several advantages. Firstly, there are many similarities between the mouse and human genome. Its genome can be easily and extensively modified (Evans, 2001). Furthermore, the easy availability of transgenic and knockout strains means that a multitude of factors contributing to disease can easily be studied with custom-made models (Moran et al., 2016). Secondly, mice models can be purpose bred and housed affordably. With a short lifespan, it becomes an easy model to measure effects of ageing (Jucker, 2010).

2.1.1. Rodent handling

Experiments were carried out according to the UK Home Office and the University of Oxford guidelines. Male C57/BL6 mice (Charles River) between the ages of 6-10 weeks were used in all experiments.

2.1.2. Generation of slices

The experiments undertaken in this thesis were all carried out in acute coronal brain sections. *Ex vivo* slices offer several advantages. The primary advantage of *ex vivo* studies is that these slices allow for experiments to be carried out under controlled conditions while still maintaining physiological structure and connections.

Slices preserve the tissue architecture of regions as well as maintain intact local synaptic function while still allowing for the precise control of environmental conditions such as temperature as well as physiological conditions such as ionic composition, and pH (Cho, Wood, & Bowlby, 2007). It also allows for control for site and speed of drug delivery. *Ex vivo* work makes for more robust experimentation as replication becomes easier.

There is evidence to show that, several manipulations, both pharmacological and genetic that have changed behaviour *in vivo* have been replicated in slices. Ex vivo slices simulate important features of *in vivo* pathologies and therefore allow for evaluation of pathologies without the complication of brain penetration, animal discomfort and metabolic instability that come with *in vivo* experimentation (Cho et al., 2007).

Drug application is also more reproducible and lacks the complications associated with variations in concentrations due to metabolism *in vivo.* Finally, various anatomical structures are easily accessible and differentiable making electrode placement more accurate (John & Jones, 2007). These characteristics also enables them to act as a good bridge between *in vivo* and *in vitro* experiments.

Measurement and manipulation of DA release can be measured *in vivo.* However, given that in this thesis the questions I ask pertain to interaction of D2 receptors and L-type calcium channels in DA release at the terminals, *ex vivo* slices provide a simple yet robust system. The use of slices reduces the complexity as well as the animal suffering related to *in vivo* work.

In the case of the meso-striatal dopamine system, a key concern is that coronal sections will sever the afferents from the mid-brain thereby impacting DA release

and measurement in the striatum. However, while the afferents are indeed severed, the axons remain. DA axons have been shown to release DA for up to 9 hours upon stimulation despite being severed from their cell bodies (Palij et al., 1990).

The animals were sacrificed using cervical dislocation. The brain was removed quickly and transferred to an ice-cold buffer solution containing HEPES. The HEPES solutions contained in mM: 3.3 HEPES sodium salt, 6.7 HEPES acid, 2 $MgSO_4.7H_2O$, 1.25 KH_2PO_4, 5 KCl, 20 $NaHCO_3$, 120 NaCl, 10 glucose and 2.4 $CaCl_2$.

Coronal sections of 300 µM thickness were made containing both dorsal and ventral striatum using a vibratome (Leica VT1200S). Slices were incubated in the HEPES buffer for an hour at room temperature and then used for experiments.

2.2. Measuring DA release

Dopamine (DA), a catecholamine is easily oxidized and reduced. A triangular waveform (-700 mV to +1300 mV) is scanned across the Carbon Fibre Microelectrode vs a reference Ag/AgCl electrode at a scan rate of 800 V/s (Figure 2.2). The oxidation of DA results in the loss of two electrons by DA to the CFM which is proportional to the amount of DA detected. Dopamine is oxidised to dopamine-*o*-quinone and can be reduced back to dopamine (Figure 2.1).

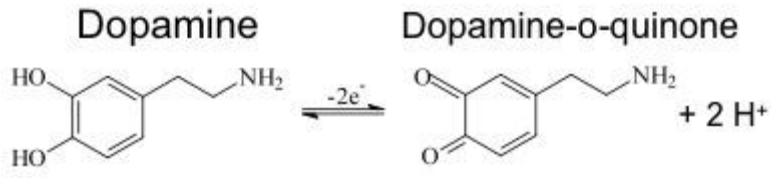


Figure 2.1. Dopamine redox reaction: Dopamine is oxidised to Dopamine-*o*-quinone which produces two-electrons and can be reduced back to dopamine.

2.3. Methods in FCV to measure DA release

The release of endogenous DA in acute brain slices was used to investigate the factors that control DA release using fast scan cyclic voltammetry (FCV).

2.3.1. Principals of voltammetry

The brain has several electroactive substances that undergo a redox reaction. Fast scan cyclic voltammetry (FCV) works on the principle of measuring the current generated from the oxidation and reduction of such electroactive substances upon the application of a stable potential. FCV measures the release of three monoamines in the brain; dopamine (DA), norepinephrine (NE) and serotonin (5-HT) (John & Jones, 2007)

The chemical identity of the substance can be identified using the location of the peaks on the voltage axis as a fingerprint. The ratio of the oxidation and reduction peak as well the number of peaks allow for the identification of the detected substance (Kawagoe, Zimmerman, & Wightman, 1993) (Figure 2.2). Repetitive cyclic voltammograms allows for the sampling of changes in composition of the solution in a time dependent manner allowing for high spatiotemporal resolution and the measurement of dynamic events. The interval between two consecutive voltammograms is usually 10 times the duration of the scan to allow for the tissue to

settle to its original state. The fast scan rate results in a background which is recorded before the stimulus and then subtracted from the response (O'Neill et al., 2017) (Figure 2.3).

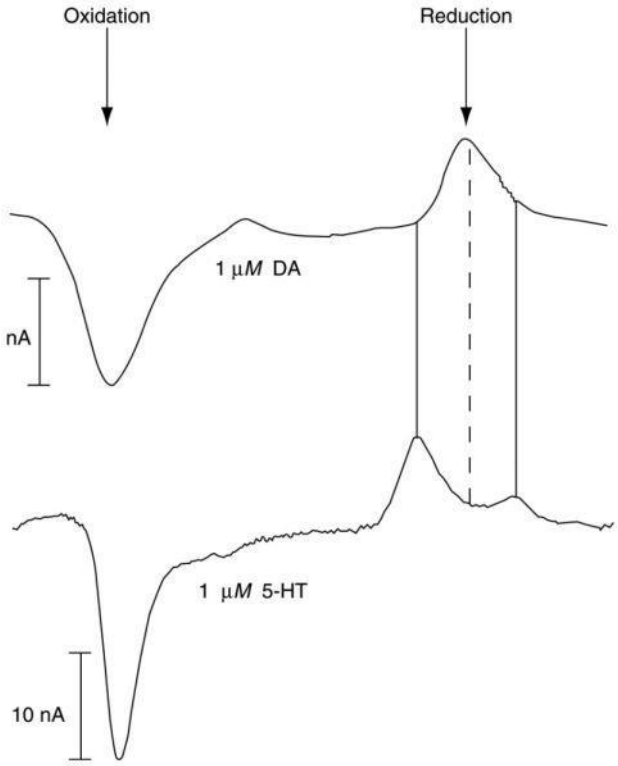


Figure 2.2. Voltammograms of different neurotransmitters: Dopamine (1 μ*M*) voltammograms (current vs. voltage curves) show one oxidation and one reduction peak. Serotonin (1 μ*M*) voltammograms show one oxidation and two reduction peaks. Voltammograms were obtained using a triangular waveform. Note that the reduction peaks of DA and 5-HT occur at different potentials. (From John & Jones, 2007).

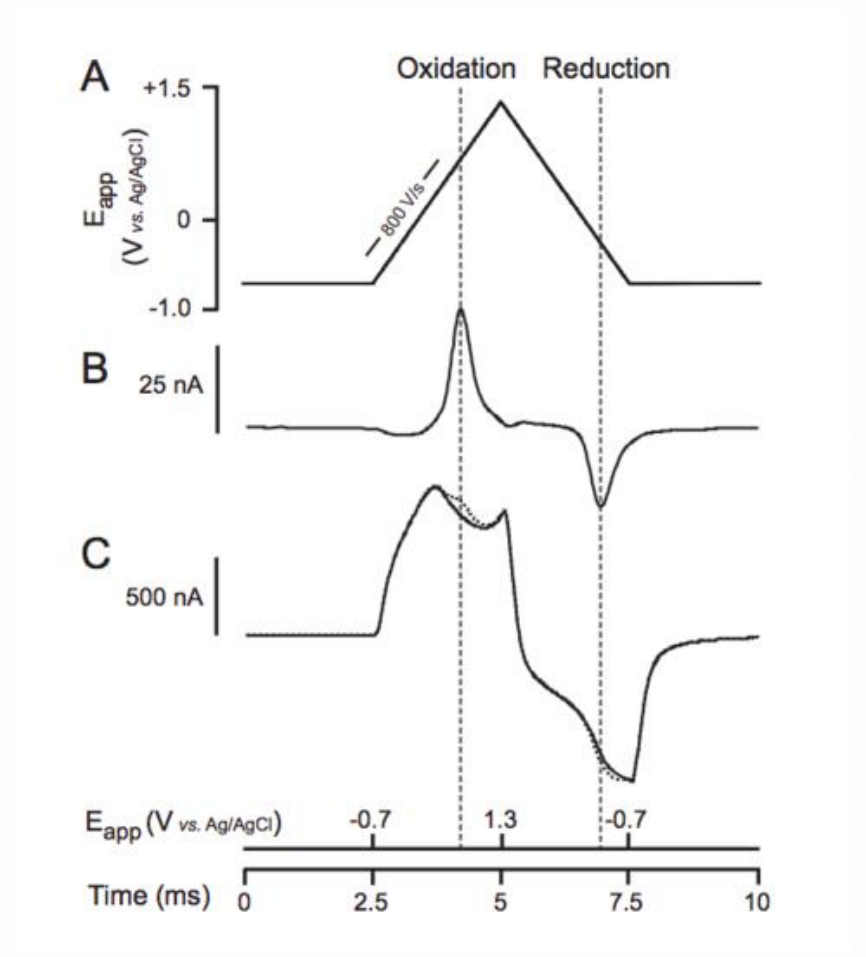


Figure 2.3. Fast scan cyclic voltammetry waveform: Fast-scan cyclic voltammetry (FCV) voltage waveform and dopamine (DA) voltammograms. (**a**) Typical triangular voltage waveform applied to a carbon-fiber microelectrode with FCV. (b) Voltammogram for 5 μM DA obtained by subtracting the background current in aCSF alone from that obtained in the presence of DA in panel **C**. **(c**) Background current generated at a carbon-fiber microelectrode with FCV in the absence (*solid-line*) and presence (*broken line*) of 5 μM DA. (Patel & Rice, 2013).

Ex vivo FCV has the advantage of being able to control the physiological environment and other parameters such as ionic compositions, pH and temperature. It also allows for spatial resolution and monitors sub-second changes to DA release. Finally, FCV offers a relatively stable method for measurement of DA over substantial periods of time (Robinson, Venton, Heien, & Wightman, 2003).

2.3.2. Preparation of carbon fibre microelectrodes

Microelectrodes made from carbon fibre were introduced nearly thirty years ago and have since become the most popular sensor for neurotransmitters (Huffman & Venton, 2009). The use of carbon fibres poses several advantages that make them biologically compatible. Carbon fibres are non-toxic to cells. Furthermore, with smaller tip diameter, usually less than ten micrometres, they cause less tissue damage (Ewing et al., 1982). Finally, their electrochemistry is well characterised, and they are known to have good electrochemical properties (Cahill et al., 1996). It is important to note that carbon fibre microelectrodes can only make extracellular recordings. The diameter of these electrodes falls within the µM range while synapses are between 20-40 nM.

Carbon fibre micro-electrodes (CFM) are produced in house and usually used for a single experiment with a new electrode used for a day of experiments. Single strands of carbon fibres are inserted and threaded through borosilicate glass capillary tubes, immersed in acetone. These carbon fibres are epoxy free to increase sensitivity to DA (Figure 2.4).

After threading of the carbon fibre, the acetone is dried through evaporation, the glass capillary tube is pulled in a vertical electrode puller to about 10 cm (Narishige, Japan). This results in tight seal formed around the carbon fibre protruding from one

of the capillary tubes. The electrode with the carbon fibre is used to produce the electrode. The exposed tip of the carbon fibre is cut to 50-100 µm under the microscope.

An insulated copper wire with some exposed uninsulated wire is coated with silver paint and inserted into the glass capillary. This forms a connection between the wire and the carbon fibre. The wire is held in place with glue. At the point of the experiment, the electrode is held by micromanipulators.

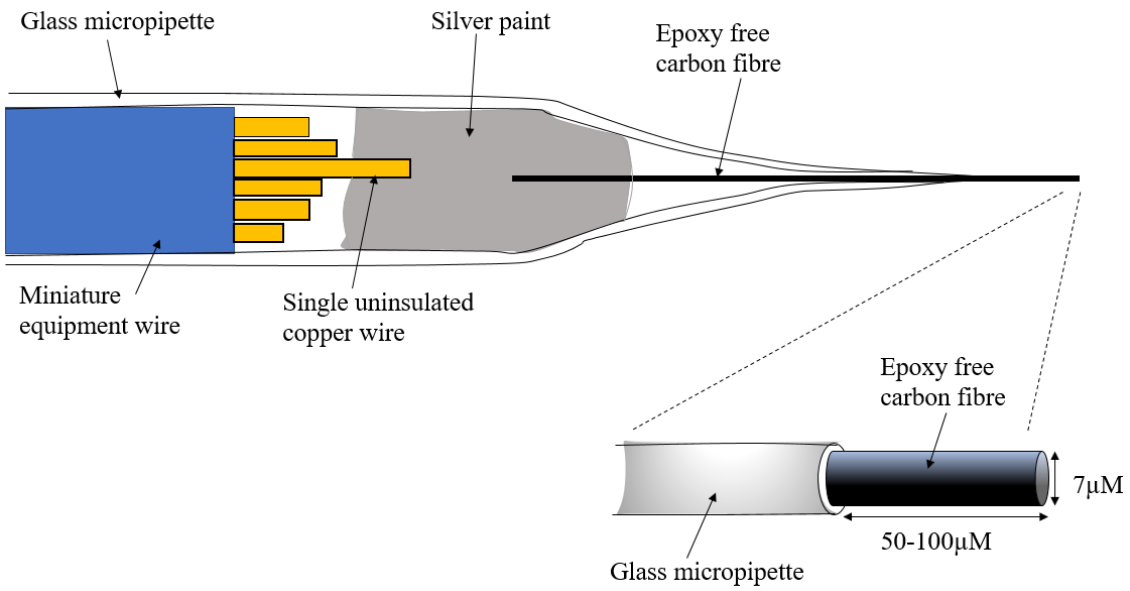


Figure 2.4. Carbon fibre microelectrode: Cross section of CFM shown the encasement of a single carbon fibre in a glass capillary. The seal is tight around the carbon fibre and a single uninsulated copper wire acts as the connection between the carbon fibre and the recording circuit (Stamford, 1995).

2.3.3. Evoking DA release

Acute striatal slices were transferred to recording chamber and super-fused with aCSF solution. Slices were held in place using silver pins. Temperature was kept between 31-33˚ as higher temperatures has been shown to increase the efficiency of the DA reuptake transporter (DAT), thereby preventing DA measurement. CFMs were inserted into the tissue into a depth of 100 µm and were left to charge for 30-60 minutes prior to any experiments being carried out. Recordings were mainly carried out in the dorsolateral striatum and the nucleus accumbens.

Recordings were made by scanning across the CFM in the triangular waveform using a Millar Voltammeter using a sampling frequency of 8 Hz. DA release was evoked using a dual protocol stimulator pulse generator triggered by a Miller voltammeter. Stimulation pulses were delivered using a stimulating electrode; a local bipolar concentric Pt/Ir electrode (FHC Inc, Bowdoin, ME, USA). This electrode was placed gently on the surface of the brain to avoid damage. The recording electrode was usually placed 100 µM away. A peri-maximal current was used to evoke the DA. This stimulating current had an amplitude of 0.6 mA and 200 µs in duration. Stimulations were repeated at intervals of 2.5 minutes. Three stimulation parameters were used to explore the thesis questions; a single pulse (1p), train pulse of 5 pulses at 5Hz (5p 5Hz) and train pulses of 5 pulses at 100 Hz (5p 100Hz).

The currents at the peak oxidation potential were measured from baseline. To calibrate the CFM, 2 µM of DA in aCSF was perfused over the CFM and the current at the peak oxidation, being directly proportional to the DA concentration, was used to convert recorded currents into DA concentrations.

2.4. Drugs and solutions

All drugs were obtained from Sigma-Aldrich or Tocris Biosciences. Drugs were made into stock aliquots in distilled water or dimethyl sulfoxide (DMSO) and stored at -20˚ till use. Immediately prior to the experiment, stock aliquots were diluted to the necessary pharmacological concentrations and added to the aCSF before being superfused over the slice. Drug concentrations used in the experiments were chosen based on previous studies as well as in accordance with dose-response curves.

In all experiments, the influence of cholinergic interneurons was blocked using 1 µM DhβE. Cholinergic interneurons (ChI) greatly influence DA release in the striatum. ChIs exhibit burst and pause changes with the pauses reducing DA release probability (Rice and Cragg, 2004; Threlfell and Cragg 2011). ChIs have also been shown to drive DA release without activity in the DA neurons (Ding et al., 2010). To remove these confounding effects and simplify the factors influencing DA release, 1 µM DhβE was added to block nAChRs in the striatum in all experiments.

Pharmacological agents were bath-applied to slices. 1 µM dihydro-ß-erythroidine (DHßE) was used throughout to prevent confounding effects of nicotinic acetylcholine receptors. 5 µM cocaine was used as the DAT inhibitor, 1 µM L-741626 was used as D2R antagonist and 5 µM isradipine was used as L-type VGCC blocker. Drugs applied under control conditions were superfused over the slice for at least 15 minutes and a stable baseline obtained before test drugs were applied. Test drugs were applied till the termination of the experiment.

2.5. Analysis of data

All experiments were replicated at least 3 times (*N*=3). Replicates were different slices from at least 3 different animals. The transients of evoked DA ($[DA]_o$) shown in figures were the average traces from across different experimental days. To ensure easy comparison, data are presented normalised to the mean control $[DA]_o$ under 1 pulse (1p) stimulation. This mean control $[DA]_o$ is calculated by averaging 4 transients under control 1p conditions. Transients after the addition of the test drug as well as after changes to stimulation frequency protocols are then represented normalised to this mean $[DA]_o$ 1p under control conditions. The effect of the test drug on peak $[DA]_o$ in all experiments was assessed by obtaining the mean peak of the last 4 transients and comparing it to mean peak under control conditions. It should be noted that all the outcomes reported in this thesis are the same as when raw data are used.

Transients were compared using a 2-way ANOVA, usually the effect of drug and time as independent variable and evoked $[DA]_o$ as dependent variable unless otherwise stated. When several stimulation protocols were used, 2-way ANOVA's were undertaken comparing evoked transients before and after the addition of the drug for each stimulation parameter separately i.e. transients evoked before and after the addition of the drug at 1p were analysed using a 2-way ANOVA and not compared using an ANOVA to the transients evoked with train stimulations.

Peak DA release under pre and post drug conditions with single stimulation protocol were analysed by plotting transients and then comparing the peak normalised DA release using t-tests. Data are expressed as mean ± standard error of mean (SEM) unless mentioned. Analysis of transients was made using a 2-way ANOVA followed

by post hoc multiple comparison t-tests (Sidak's and Tukey's). The level of significance for all statistical analysis was set at $p < 0.05$. All graphs were obtained using GraphPad Prism 4.

3. Results

Rationale for study

L-type voltage gated calcium channels (VGCCs) have been shown to contribute to evoked dopamine (DA) release in the CPu. The addition of isradipine, an L-type specific calcium channel blocker of the dihydropyridine class significantly decreased evoked DA release to 75% of controls in the CPu (2-way ANOVA: $F_{1,30}$=20.38; *P<0.001* from Brimblecombe *et al.,* 2015) (Figure 3.1A left transient) (Brimblecombe *et al.,* 2015). This effect of isradipine was found to be occluded in the CPu in the presence of cocaine (Figure 3.1A right transient) (Brimblecombe, unpublished data Cragg lab).

Cocaine acts by blocking the dopamine transporter (DAT) which mediates the reuptake of DA. This leads to an increase in the release of DA as well as slower re-uptake resulting in increased extracellular DA. Extracellular DA is known to activate D2 autoreceptors which play an important role in modulating re-release of DA. One mechanism through which the re-release is modulated is through the βγ-mediated inhibition of VGCCs.

Given that the block of DAT by cocaine would result in increased $[DA]_o$ which in turn would result in increased activation of D2Rs and knowing that D2Rs have been shown to inhibit the activity of VGCCs, in this thesis, we investigated the hypothesis that the occlusion of the isradipine effect in the presence of cocaine in the CPu was due to inhibition of these channels by the D2Rs.

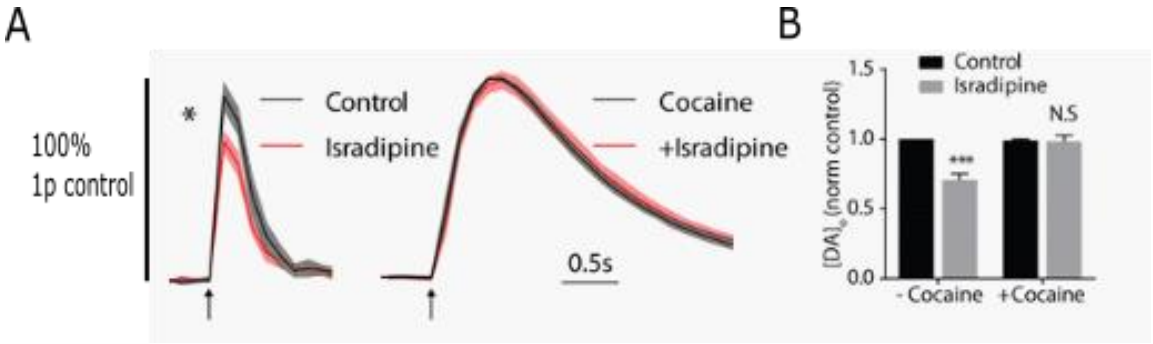


Figure 3.1. The effect of isradipine is occluded in the presence of cocaine. A) Mean transients in the absence of cocaine (left) and in the presence of cocaine (right). Evoked $[DA]_o$ (normalised to peak max pre-isradipine condition) ± SEM vs time. 2-way ANOVA: $*P < 0.05$ B) Mean peak ± SEM (normalised to 1p under pre-isradipine conditions(control)) without cocaine and in the presence of cocaine. N=3 (Brimblecombe, unpublished data, Cragg Lab).

3.1. L-type VGCCs contribute to evoked DA release in the CPu-corroboration of published study

All experiments were conducted in the presence of nAChR block (1 µM DHβE) to remove the potentially confounding influence of ACh on DA release. Release of DA, like all other neurotransmitters, is driven by pre-synaptic VGCCs. The role of L-type VGCCs in the release of DA in the dorso-lateral CPu, previously shown by *Brimblecombe* et al. (2015), was replicated and confirmed (Figure 3.2). The bath application of 5µM isradipine to block L-type VGCCs in the CPu resulted in a significant decrease in electrically evoked $[DA]_o$ release to ~70% of that under pre-isradipine control conditions as analysed using a 2-way ANOVA (Figure 3.2A; 2-way ANOVA, independent variables; time and drug, main effect of drug: $F_{1,264}=38.04$, $p<0.0001$). Raw absolute concentrations of [DA]o under control conditions and after the addition of Isradipine were compared using a t-test and peak [DA]o release was found to have significantly decreased after the addition of Isradipine (Figure 3.2C). This decrease is equivalent to published findings (Brimblecombe *et al.,* 2015).

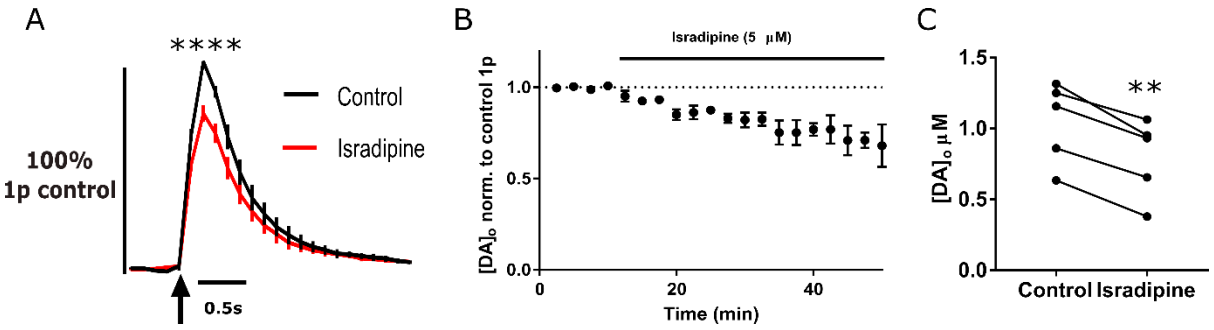


Figure 3.2. L-type VGCC modulates evoked $[DA]_o$ in CPu: Control conditions are all carried out in the presence of 1µM DhβE. (A) Mean transient profile of evoked $[DA]_o$ ± SEM for 1p vs time. Data are normalized to 1p evoked $[DA]_o$ under DHβE. Control conditions (black lines) and isradipine (red). 2-way ANOVA: ****P<0.0001 (B) Wash on profile with peak max values normalised to 1p evoked $[DA]_o$ under control conditions. The duration of isradipine wash on is shown by the black bar. (C) Mean peak (uM) ± SEM evoked for 1p for each experiment under control conditions and after isradipine wash on. Two-tailed paired *t*-Test: ** t_4= 7.922, P= 0.0014; n=*5*.

3.2. Do D2 receptors modulate L-type function in the CPu?

The lack of the isradipine effect in the presence of cocaine in CPu as seen by Brimblecombe (Figure 3.1) was hypothesised to be due to the activation of D2 receptors. By blocking the DAT, cocaine slows down the reuptake of DA from the extracellular space thereby increasing extracellular DA. We hypothesise that this increase of extracellular DA results in a greater activation of D2 receptors which modulate L-type VGCCs via a βγ-mediated inhibition. This inhibition results in the lack of an isradipine effect in the presence of cocaine. In order to test this hypothesis, we first tested whether D2 receptors modulate L-type function in the CPu.

We began by exploring the extent of D2 activation and modulation of DA release in the CPu. D2 activation occurs after the initial release of DA, therefore, it can be hypothesised that the greatest effect of the antagonist will be seen after a second stimulation. The activation of D2 receptors in the dorsal striatum have been described to occur after 200 ms with maximum activation occurring at ~ 550ms. Therefore, any effect of the D2 antagonist would not be expected to be evident until the D2 receptors are active which will only occur under longer stimulation protocols (Philips *et al.,* 2002). However, it is also possible that the downstream process of D2 activation is sufficiently long-lasting to be maintained between our 2.5 min stimulation interval. In order to thoroughly examine the modulation of D2Rs on DA release, we used a range of protocols to explore effects of D2R antagonism; 1p and 5p trains were used to explore the effect of D2Rs on DA release. A low (5Hz) and a high (100Hz) frequency train stimulation protocol was used. It is important to note that these two stimulation protocols, other than differing in frequency also differ in the

duration of stimulus with the 5Hz protocol lasting 800 ms while the 100Hz protocol lasting 40 ms.

D2R antagonist, L741626 (1 µM), washed on after obtaining a stable baseline in DHβE (1µM), had no significant effect on *peak* evoked $[DA]_o$ under all stimulation frequencies (Figure 3.3B; 2-way ANOVA, $F_{1,12}$=1.112, *p=0.3125*).

However, when the transients were compared; under control conditions and after the addition of L741626, a significant effect of the D2 antagonist was revealed i.e. evoked $[DA]_o$ at various points over the time course of the transients were significantly different between the two conditions. Under train stimulation protocols at 5Hz and 100Hz, the addition of the D2 antagonist had a significant effect on DA release revealed by a 2-way ANOVA undertaken for each stimulation separately (Figure 3.3A; 2-way ANOVA for 5p at 5Hz, main effect of drug: $F_{32,132}$=78.64, *p<0.0001*; 2-way ANOVA for 5p at 100Hz, main effect of drug: $F_{1, 132}$=26.07, *p<0.0001*).

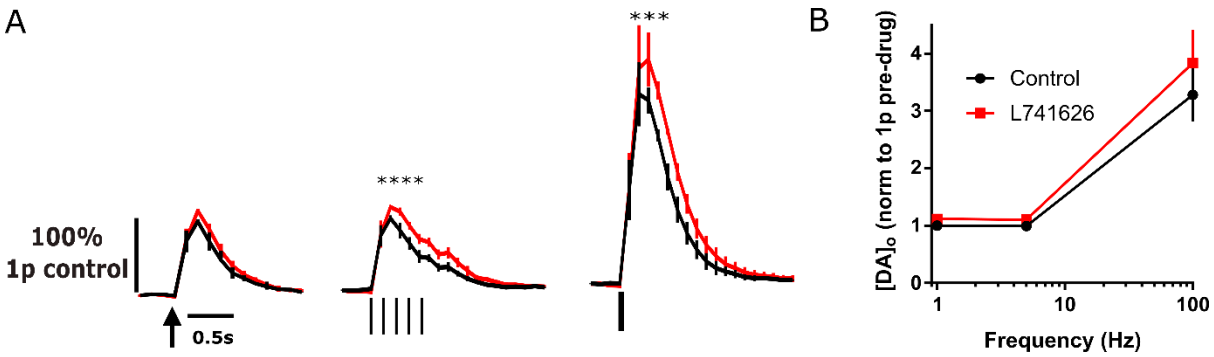


Figure 3.3. D2 receptors modulate DA release in the CPu under train stimulations but do not have a significant effect on peak DA release: Control conditions are carried out in the presence of 1μM DhβE (A) Mean transient profile of evoked $[DA]_o$ ± SEM under different stimulation protocols. Data are normalized to 1p evoked $[DA]_o$ under control conditions. Control conditions (black) and after L741626 (red). From L-R, evoked normalized transient at 1p, 5p at 5Hz (centre) and 5p at 100Hz. 2-way ANOVA done for each frequency stimulation separately: ****$P < 0.00001$ *** $P<0.0001$ (B) Mean peak ± SEM under control (black) and after addition of L741626 (red). n=3

Having confirmed the modulation of DA release by D2Rs in the CPu, we then washed on isradipine in the presence of the D2R blocker to investigate the interaction between D2Rs and L-type VGCCs. D2Rs were blocked using L741626 (1 µM) to obtain a baseline and the L-type VGCCs antagonist isradipine (5 µM) was then washed on. We used a single stimulus protocol (1p) to study changes to DA release. The addition of isradipine resulted in modest but significant decrease in evoked DA release to ~75% control levels which was analysed using a 2-way ANOVA (Figure 3.4A; 2-way ANOVA, $F_{1,198}$=7.031, *p=0.0087*).

However, this decrease in evoked DA release with the addition of isradipine in the presence of the D2R antagonist was of the same magnitude as the decrease in the absence of the D2R antagonist (Figure 3.4B; 2-way ANOVA, $F_{1,4}$=0.02189, *p=0.8895*). Upon comparison of the release of DA under both conditions, using a t-test we found that effects of the addition of isradipine were not significantly different between the two experimental conditions (Figure 3.4C; two-tailed T-test; t_7=0.5371, p=0.6078).

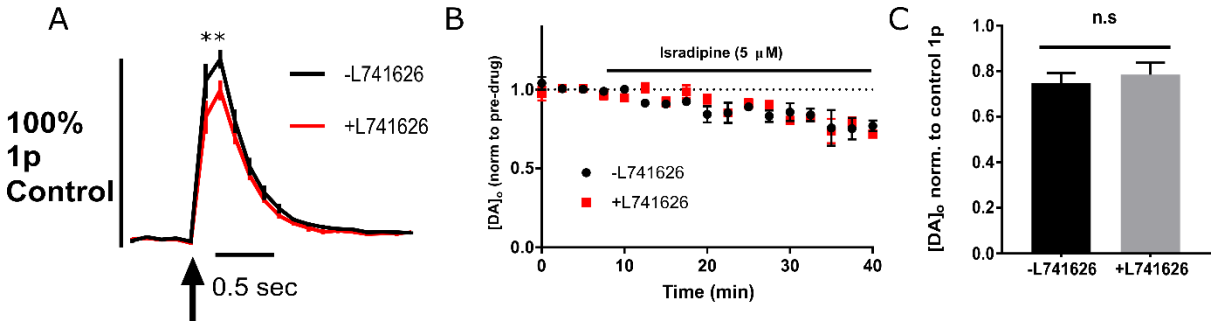


Figure 3.4. D2 does not modulate L-type function in the CPu: Control conditions are all carried out in the presence of 1µM DhβE and 1 µM L741626. (A) Mean transient profile of evoked $[DA]_o$ ± SEM at single pulse stimulation. Data are normalized to 1p evoked $[DA]_o$ under control conditions. Control conditions (black) and Isradipine (red). 2-way ANOVA: ***P*= 0.0087 (B) Wash on profile with peak max values. Wash on profile of the experiment in the absence of L741626 (black) and presence of L741626 (red). (C) $[DA]_o$ upon addition of isradipine, normalised to pre-isradipine values in the presence and absence of L741626. N=5 for experiment in the absence of L741626 and N=4 for experiment in the presence of L741626.

We concluded that while D2R antagonists do have a modest effect on DA release especially under train stimuli, there appears to be a lack of modulation of L-type calcium channels in the CPu by D2Rs.

3.3. Do D2 autoreceptors modulate L-type function in the presence of cocaine, in the CPu?

In the previous section, we found that D2Rs modestly control DA release and have a more significant effect on DA release under train stimulation. The original hypothesis set out to investigate the lack of L-type function in the presence of cocaine. Cocaine, as a DAT inhibitor, increases extracellular DA levels and therefore should result in a much greater activation of D2Rs. We tested whether the activation of D2Rs and their modulation of DA release is greater in the presence of cocaine compared to the absence of the same.

To investigate the role of D2 auto-receptors in modulating $[DA]_o$ release in the presence of cocaine, the D2 antagonist L741626 (1 µM) was washed on in the presence of cocaine. D2 activation occurs after the initial release of DA, therefore, it can be hypothesised that the greatest effect of the antagonist will be seen at and after a second stimulation. This will be best seen at lower frequency, longer stimulation protocols.

The addition of the D2 antagonist saw a trend towards the increase of *peak* evoked $[DA]_o$ but did not reach significance under all stimulation protocols as analysed by a 2-way ANOVA for each stimulation parameter separately (Figure 3.5B; 2-way ANOVA, $F_{1,12}$=4.609, *p=0.0530*). The effect of cocaine and therefore any D2 activity will be most significant not at the peak but after initial release. A 2-way ANOVA of the average transients under each stimulation parameter before and after the addition of the D2R antagonist revealed a significant effect of the D2R antagonist under the train stimulus at both 5Hz and 100Hz (Figure 3.5A; 2-way ANOVA, 5Hz: $F_{1,180}$=425.5, *p<0.0001,* 100Hz: 2-way ANOVA, $F_{1,164}$=41.33, *p<0.0001*). Unexpectedly, a significant effect of the D2R antagonist was also revealed upon

analysis of the average evoked transients with a single stimulus (1p) indicating that the DAT block by cocaine and the resultant extracellular DA was sufficient to activate D2 receptors (Figure 3.5A; 2-way ANOVA, $F_{1,164}$=46.42, *p<0.0001*).

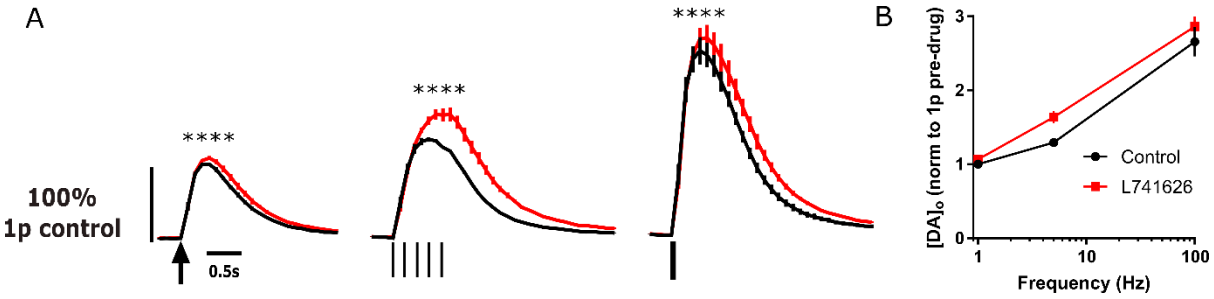


Figure 3.5. D2 receptors modulate DA release under slower frequency stimulations in the presence of cocaine in the CPu: Control conditions are carried out in the presence of 1μM DhβE and 5 μM cocaine (A) Mean transient profile of evoked $[DA]_o$ ± SEM under different stimulation protocols. Data are normalized to 1p evoked $[DA]_o$ under control conditions. Control conditions (black) and after L741626 (red). From L-R, evoked normalized transient at 1p, 5p at 5Hz (centre) and 5p at 100Hz. 2-way ANOVA: ****P<0.0001 (B) Mean peak ± SEM under control (black) and after addition of L741626 (red). n=3

Having confirmed that in that the presence of cocaine results in D2R activation, we then investigated whether this activation of D2R is greater in the presence of cocaine, as we earlier hypothesised. Since the effect of a D2R activation will not be effective at the peak but rather most striking throughout the train, we compared the transients. However, it was difficult to compare the transients with and without the effect of cocaine as cocaine has a significant effect on DA release. In order to make the comparison less complicated, under both conditions (with and without cocaine), evoked $[DA]_o$ before the addition of the D2R antagonist was subtracted from the evoked $[DA]_o$ after the addition of the D2R antagonist at every time point in the average evoked transient and plotted vs time. The magnitude of this difference was compared between the two conditions (with cocaine and without cocaine).

Analysis of the magnitude of difference between the evoked $[DA]_o$ were analysed using a 2-way ANOVA. Significant differences between the D2R activation in the presence and absence of cocaine was observed under the train stimulus of 5Hz (Figure 3.6A.2-way ANOVA, effect of D2R antagonist: $F_{1,132}$= 170.2; P<0.001). This was not so at 100Hz (Figure 3.6A 2-way ANOVA, $F_{1,132}$= 0.1864; P= 0.6667). In keeping with the unexpected D2R activation under a single stimulus found in the previous section, a significant difference in magnitude was observed under a single pulse stimulus (Figure 3.6A. 2-way ANOVA, effect of D2R antagonist: $F_{1,132}$= 32.22; P<0.001).

The 2-way ANOVA compared differences in magnitude of release under each condition at every time point. However, the effect of time once again made the data difficult to interpret. For example, in the case of the 1p stimulation protocol, a significant difference in magnitude was observed upon analysis, however the figure seems to indicate that the effect of time greatly influences that observation. Given

the complexities caused by the interaction between time and the effect of D2 block, it was difficult to interpret the data. In order to simplify the comparison, we compared the area under the curve at each frequency (Figure 3.6B). A strong interaction between the drug effects and frequency were seen and both had significant effects (Figure 3.6B. 2-way ANOVA, effect of D2R antagonist: $F_{1,12}$= 32.98; P<0.001; 2-way ANOVA, effect of frequency: $F_{2,12}$= 63.16; P<0.0001). Furthermore, a post-hoc Sidak's multiple comparisons test revealed a significant difference between the D2R effect between the two different conditions at the train stimulation of 5Hz (Post hoc test; P<0.0001).

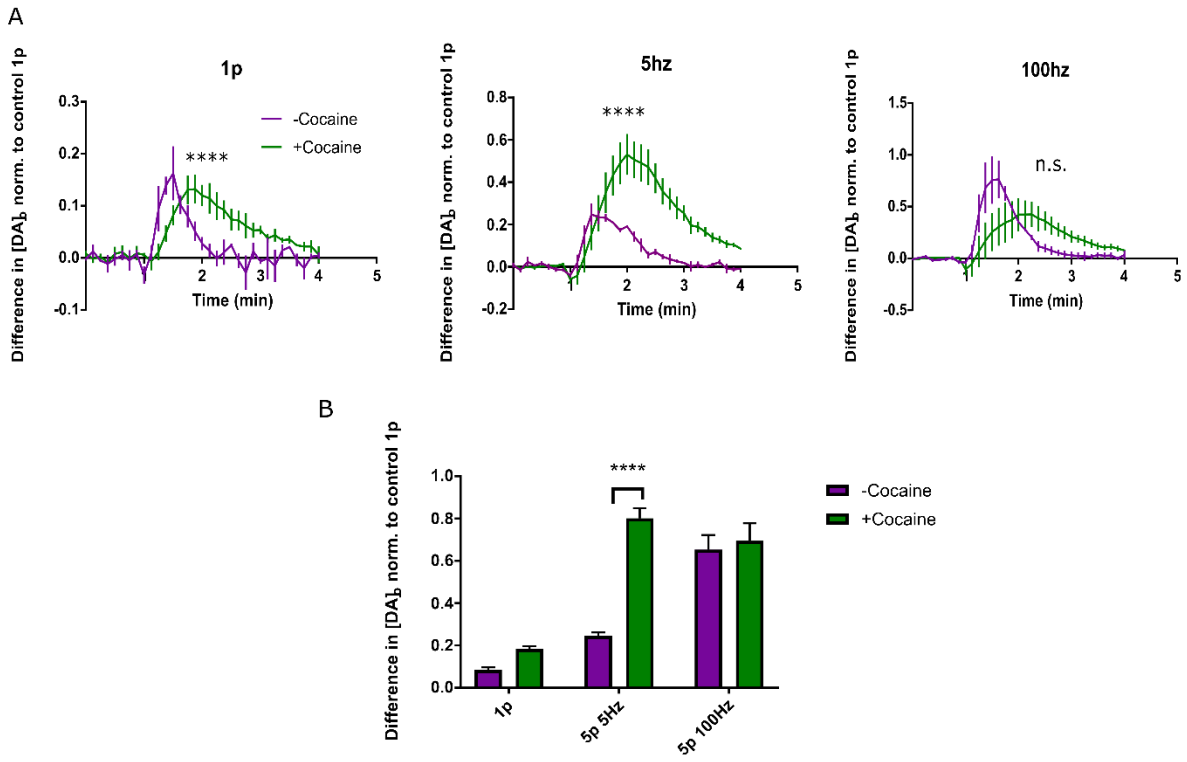


Figure 3.6. Magnitude of D2 receptor activation is greater in the presence of cocaine at single pulse and at slower train stimulations in the CPu: Difference of mean transient profile of evoked $[DA]_o$ ± SEM before and after addition of D2R antagonist in the presence and absence of cocaine. $[DA]_o$ levels are normalized to 1p evoked $[DA]_o$ under respective control conditions. From L-R, difference of evoked normalized transient at 1p, 5p at 5Hz (centre) and 5p at 100Hz. Presence of cocaine (green), absence of cocaine (purple). 2-way ANOVA for each stimulation protocol: ****$P< 0.0001$. (B) Comparison of area under the curve in the presence of cocaine (green) and absence of cocaine (purple) under each stimulation frequency; 1p (left), 5p at 5Hz (centre) and 5p at 100Hz (right). Post-hoc Sidak test ****$P< 0.0001$. n=3.

We concluded that in the presence of cocaine, there is greater D2R activation and the most significant difference in activation occurs at 5Hz.

3.4. Is the lack of L-type function under the presence of cocaine due to D2 activation?

In the presence of cocaine, the inhibitory function of the L-type channel blocker isradipine was occluded (Figure 3.1). We hypothesised that this was due to the activation of D2 receptors modulating L-type VGCCs via a βγ-inhibition. While such an interaction was absent without cocaine, we confirmed that D2Rs are significantly more active in the presence of cocaine which can be conjectured to have a greater inhibitory effect on L-type channels. We tested whether the increase in D2R activation in the presence of cocaine was the cause of the lack of an isradipine effect.

A stable baseline was obtained in the presence of cocaine and the D2R antagonist. The L-type channel were subsequently blocked by the washing on isradipine (5 µM). Isradipine did not decrease DA release under all stimulation protocols. Instead, the addition of Isradipine resulted in a significant increase in DA release (Figure 3.7A; 2-way ANOVA, $F_{2,12}$= 133.1, p= 0.0094) this increase was seen with all frequencies of stimulation. The most significant effect was observed when a train stimulus of 5 Hz was delivered (Figure 3.7B; Post-hoc Sidak test; p=0.0279).

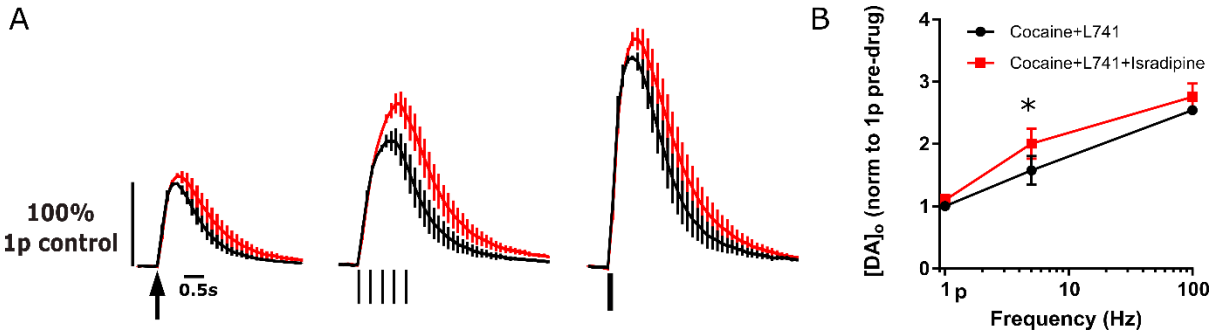


Figure 3.7. Lack of L-type VGCC modulation of evoked $[DA]_o$ in CPu under cocaine is not due to block by D2: Control conditions are carried out in the presence of 1μM DhβE, 5 μM cocaine and 1μM L741626 (A) Mean transient profile of evoked $[DA]_o$ ± SEM under different stimulation protocols. Data are normalized to 1p evoked $[DA]_o$ under control conditions. Control conditions (black) and after Isradipine (red). From L-R, evoked normalized transient at 1p, 5p at 5Hz (centre) and 5p at 100Hz; 2-way ANOVA; **p= 0.0094 (B) Mean peak ± SEM under control (black) and after addition of Isradipine (red). Post-hoc Sidak *P= 0.029 for 5Hz. n= 3

The lack of L-type VGCCs inhibition led us to conclude that the lack of L-type modulation under cocaine seen by Brimblecombe was not due to inhibition by D2Rs.

3.5. Do D2Rs modulate L-type function in the NAc in the absence of cocaine?

It has been well established that the availability and parameters modulating DA release can vary between different striatal regions (Cragg *et al.,* 2002). This is mediated by several drivers of DA release and re-uptake, including differences in VGCCs involved, the concentration and activity of the DAT as well as the D2R function (Rice *et al.,* 2011).

Brimblecombe et al. (2015) showed that evoked $[DA]_o$ in the NAc was not mediated by L-type VGCCs, a result that was replicated for *n*= 1 (Figure 3.8). The same study also found that the L-type function could be unmasked when extracellular Ca^{2+} concentration was increased; therefore, we are interested in potential physiological regulators that can silence and recruit LTCC function. Furthermore, a recent paper by *Marcott* et al. (2018) showed regional heterogeneity in D2 activation and sensitivity to DA between dorsal and ventral D2Rs. A difference in activation kinetics of D2R comes as no surprise given the regional heterogeneity. The time window in which D2R are activated in the NAc is longer than that of D2R in the CPu. Much like the CPu, D2R in the NAc begin to activate 200 ms after initial DA release. However, the peak autoreceptor activation occurs ~ 700ms (Phillips *et al*., 2002).

There appear to be several physiological clamps on the L-type function in the NAc and we explored the potential for D2R to limit L-type VGCC function in NAc.

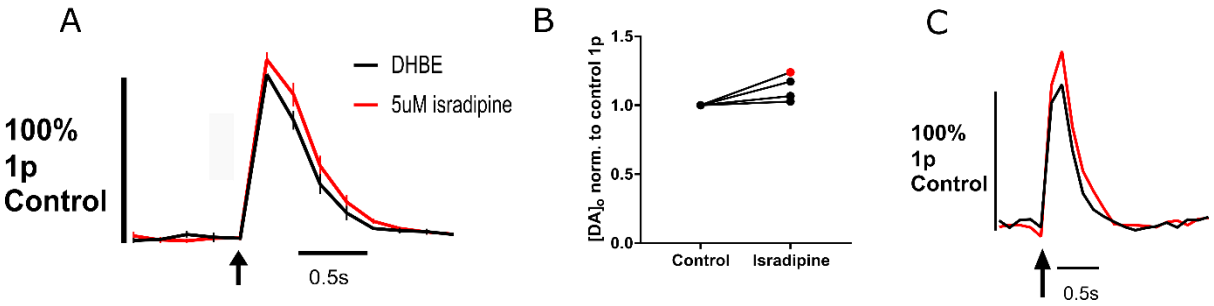


Figure 3.8. L-type VGCC modulates evoked $[DA]_o$ in NAc: Control conditions are all carried out in the presence of 1μM DhβE. (A) Mean profiles of [DA]o ± SEM vs. time evoked by 1p in NAc in controls or the presence of isradipine (5 μm). Data are normalized to 1p peak $[DA]_o$ evoked in control conditions. Control conditions (black lines), isradipine (grey lines) (From Brimblecobe *et al.,* 2015). (B) Mean peak (μM) ± SEM evoked for 1p for each experiment under control conditions and after Isradipine wash on. All data are normalised to 1p control conditions. Data from Brimblecombe *et al.,* (2015) shown in black, data of n=1 obtained as part of this thesis shown in red. (C) Mean transient profile of evoked $[DA]_o$ ± SEM at 1p obtained as part of this thesis. Data are normalized to 1p evoked $[DA]_o$. Control conditions (black) and Isradipine (red).

D2 receptors were blocked using L741626 (1 µM) and the L-type VGCCs blocker isradipine (5 µM) was washed on. Under a single stimulation protocol (1p), the addition of 5 µM isradipine resulted in significant decrease in peak evoked DA release to 80% of control release as analysed by a 2-way ANOVA (Figure 3.9.A. 2-way ANOVA, $F_{1, 109}$=31.54, *p<0.0001*). The raw evoked $[DA]_o$ under both conditions were compared and analysed using a t-test (Figure 3.9B).

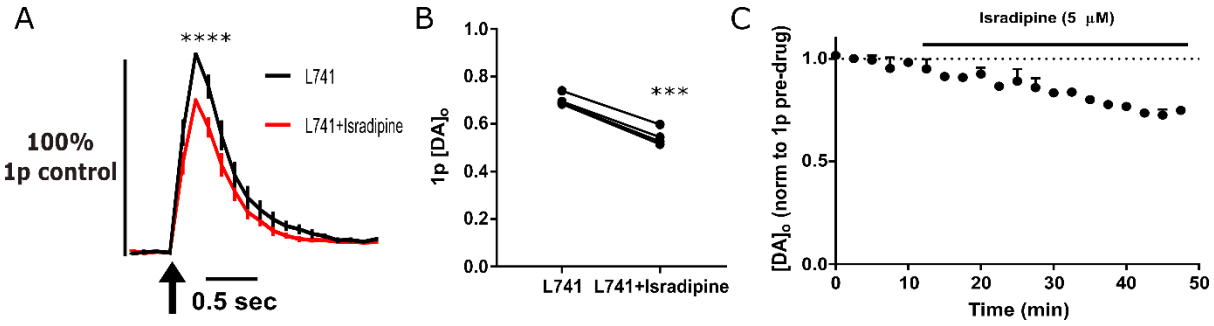


Figure 3.9. D2 modulates L-type function in the NAc: Control conditions are all carried out in the presence of 1µM DhβE and 5 µM L741626. (A) Mean transient profile of evoked $[DA]_o$ ± SEM at single pulse stimulation. Data are normalized to 1p evoked $[DA]_o$ under control conditions. Control conditions (black) and Isradipine (red). 2-way ANOVA: ****P<0.0001 (B) Mean peak (uM) ± SEM evoked for 1p for each experiment under control conditions and after addition of isradipine. Two-tailed *t*-Test: ***P< 0.0001(C) Mean normalized peak $[DA]_o$ ± SEM evoked by single pulse during wash on. n=4

The unexpected isradipine effect unmasked L-type VGCC activity in the presence of a D2R blocker suggesting that the lack of L-type VGCC activity in the NAc seen by Brimblecombe *et al.,* (2015) could be attributed to a D2R inhibition of these channels. We explored whether D2R activity is significantly different between the CPu and the NAc.

3.6. Is D2Rs modulation of DA release greater in the NAc compared to the CPu?

Given the unexpected modulation of L-type VGCCs by D2 receptors in the NAc which does not occur in the CPu, we investigated whether there are significant differences in D2R function between the CPu and the NAc.

We began by testing D2 receptor activation in the NAc under various stimulation protocols. The D2 antagonist L741626 (1 μM) was washed on after obtaining a stable baseline with DhβE (1 μM). D2 receptors had no significant effect on the modulation of peak evoked DA under all stimulation frequencies (Figure 3.10B; 2-way ANOVA, $F_{1,12}$=1.803, *p=0.2042*). A two-way ANOVA however revealed significant D2 inhibitory effects at the longer train 5Hz stimulation protocol (Figure 3.10A; 2-way ANOVA, $F_{1,132}$=23.64, *p<0.001)* as well as at 100Hz train protocol (Figure 3.10A; 2-way ANOVA, $F_{1,132}$=28.5, *p<0.0001).*

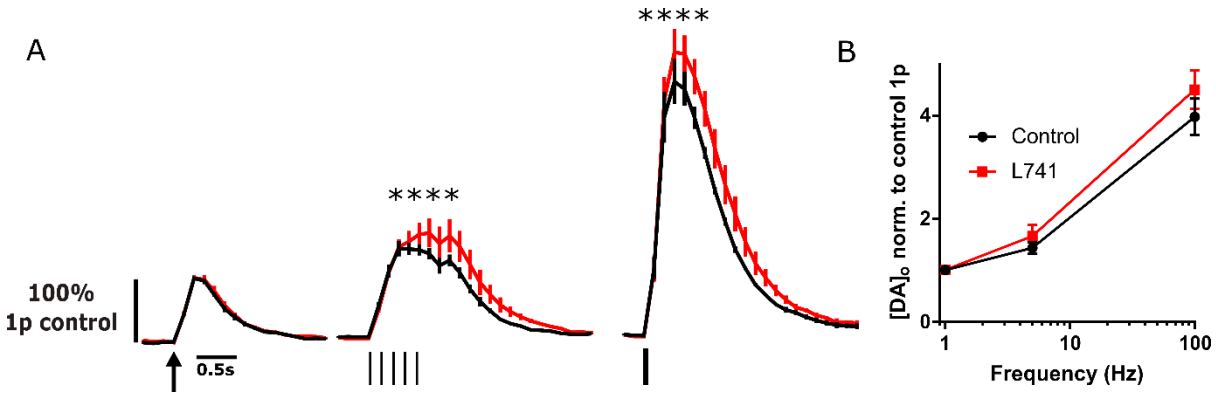


Figure 3.10. D2 modulates DA release in the NAc: Control conditions are all carried out in the presence of 1μM DhβE. (A) Mean transient profile of evoked $[DA]_o$ ± SEM under different stimulation protocols. Data are normalized to 1p evoked $[DA]_o$ under control conditions. Control conditions (black) and after Isradipine (red). From L-R, evoked normalized transient at 1p, 5p at 5Hz (centre) and 5p at 100Hz. 2-way ANOVA: ****P<0.0001 (B) Mean peak ± SEM under control (black) and after addition of Isradipine (red). n=3

To investigate whether D2R activity was significantly different between the CPu and the NAc we compared the difference in DA release between the two regions over the course of the stimulus duration before and after the addition of the D2 antagonist. The magnitude of the difference in DA release was correlated to greater D2R activity. We analysed the difference between evoked DA release at every time point over the entire stimulation for all stimulation protocols. This difference in DA release was compared between regions by plotting vs time.

The magnitude of the difference in release was found to be significantly different between the two regions at slow train stimulations of 5Hz but this results was difficult to interpret as there was a significant interaction with the effect of time (Figure 3.11A;

2-way ANOVA for regional differences, $F_{1,132}$=14.05, p=0.0003; 2-way ANOVA for time, $F_{32,132}$=6.082, p<0.0001). There was no significant difference in the D2R activation between the two regions under the single stimulus and the train stimulation at a 100Hz (Figure 3.11A; 2-way ANOVA for 1p, $F_{1,100}$=1.347, p=0.2486 and 2-way ANOVA for 5p at 100Hz, $F_{1,132}$=1.958, p=0.1641).

Given the significant interactions between time, it was difficult to interpret the data. In order to simplify the comparison, we compared the area under the curve at each frequency (Figure 3.11B). There was a significant difference in the D2R antagonist effect between the two regions (Figure 3.11B. 2-way ANOVA, $F_{2,12}$= 57.34; P=0.0490). A significant effect of frequency was also observed (Figure 3.11B; 2-way ANOVA, region × frequency interaction $F_{2,12}$= 57.34; P<0.0001) A post-hoc Sidak's multiple comparisons test revealed a significant difference between the D2R effect under the two different regions at the train stimulation of 5Hz (Post hoc test; P=0.0485)

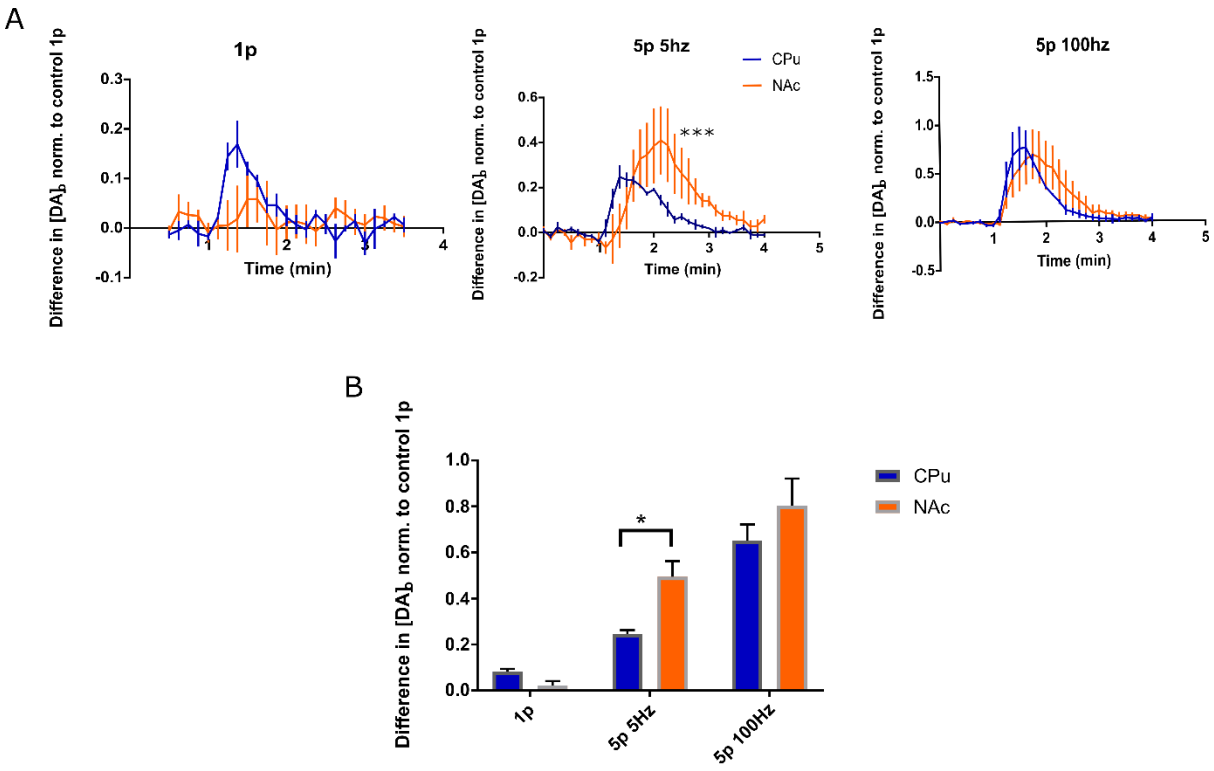


Figure 3.11. Difference in DA release between CPu and NAc through stimulation: (A) The difference between DA release after addition of L741626 in the CPu and NAc. Data are normalized to 1p evoked $[DA]_o$ under control conditions. CPu (Blue) and NAc (orange). From L-R, evoked normalized transient at 1p, 5p at 5Hz and (centre) and 5p at 100Hz. 2-way ANOVA: ****P*=0.003. (B) Comparison of area under the curve at different stimulation protocols between the CPu (blue) and the NAc (orange); 1p (left), 5p at 5Hz (centre) and 5p at 100Hz (right). Post-hoc Sidak **P= 0.0485.* n=3.

D2R activation between the two regions within the paradigms of this experiment appear to only be significantly different at slow train pulse stimulations.

4. Discussion

4.1. Overview

Striatal dopamine (DA) release is regulated by several key factors including voltage gated calcium channels (VGCCs) driving DA release, the re-uptake kinetics modulated by the dopamine reuptake transporter (DAT) and the regulation of re-release by the D2 autoreceptors (Rice et al. 2011). Given the importance of understanding the changes to striatal DA release in understanding neurodegenerative conditions like Parkinson's disease, I attempted to investigate the interactions between the D2 autoreceptors (D2Rs) and L-type VGCCs in modulating the release of DA in the striatum. This chapter will consolidate and discuss the results of my experiments and attempt to contextualise these findings in relation to what has been previously described in the literature. This thesis has identified several layers of modulation and control of L-type VGCC modulation of DA release with variations across striatal subregions.

4.2. Main findings

The release of DA in the striatum occurs through the exocytosis of vesicles. Release is also action potential dependent and catalysed by a variety of VGCCs (Brimblecombe et al*.,* 2015). L-type VGCCs, traditionally thought not to play a role in neurotransmitter release, have been shown to modulate the release of DA in the CPu (Brimblecombe, et al., 2015). The addition of the L-type VGCCs antagonist, isradipine, in the dorsal striatum was found to decrease release by up to 30%. Unpublished data from the Cragg lab showed that this L-type function was occluded in the presence of the dopamine reuptake transporter (DAT)-blocker cocaine. The main aim of this project was to investigate whether the occlusion of L-type VGCC

function in the presence of cocaine was due to inhibition of L-type VGCCs by D2 receptors.

In brief, confirming that D2R activity is heterogeneous across the striatum, we showed that D2R modulation of DA release is greater in the NAc under 5p trains stimulations, but at a single pulse stimulation, CPu had greater D2R activity. We also show that D2R activity and modulation of DA release is greater in the presence of cocaine and significantly so at 5p train stimulations in the CPu. We compared the interaction of D2Rs with L-type VGCCs in the presence and absence of cocaine in the CPu. We found no interaction between D2Rs with the L-type VGCC in the absence of cocaine. In the presence of cocaine, the addition of L-type blocker unexpectedly increased DA release. We concluded that D2Rs do not interact with, or limit, L-type function in the CPu. However, this is not true in the NAc where we found that D2Rs limit the L-type VGCC function.

4.3 Cocaine increases D2-autoreceptor function on DA axons in CPu

In the presence of cocaine, we found that D2R activation is greater in the CPu. D2Rs function is significantly greater at longer time stimulation protocols, especially at a train stimulation at 5Hz.

Cocaine not only increases extracellular DA by decreasing the rate of reuptake, it is also thought to increase DA release via modulation of reserve pool of DA vesicles (Ewing et al.,1983; Venton et al., 2006). DAT knockout experiments have demonstrated a hyperdopaminergic tone in the striatum (Jones et al., 1995). Increase in extracellular DA appears to result in an increased activation of D2Rs.

D2Rs decrease subsequent DA release through various GPCR-coupled mechanisms. D2R- driven inhibition of neuronal VGCCs is complex (Zamponi &

Currie, 2013). The key step is the dissociation of the Gα and the Gβγ from the receptor. Both these subunits can inhibit the influx through Ca^{2+} channels, however the mode of their action is different. While Gβγ acts by structurally inhibiting the VGCCs, Gα mediates inhibition via downstream signalling pathways that involve phosphorylation of the channels. We were unable to see an interaction between D2Rs and L-type VGCCs in the CPu without cocaine. Therefore, we added cocaine to increase D2R activation and then investigated whether there was an interaction.

4.3.1. Enhanced D2R activation does not inhibit L-type function and control of DA release

We confirmed that the presence of cocaine increases D2R activity significantly at a slower frequency stimulation. Brimblecombe (unpublished data) found that the effect of isradipine was occluded in the presence of cocaine. Given that D2Rs show increased activity in the presence of cocaine and that there is a precedence for D2R inhibition of VGCCs, we hypothesised that L-type function was occluded in the presence of cocaine due to the D2R inhibition.

We tested this hypothesis by measuring changes to DA release in the presence of cocaine, a D2R blocker and isradipine. We found that in the presence of cocaine and a D2R antagonist the effect of isradipine remained occluded. The addition of isradipine significantly increased DA release under all stimulation protocols with the greatest effect at the slower train stimulus protocols (5p at 5Hz). Lack of the expected decrease in DA release led us to conclude that the lack of L-type VGCC activity in the presence of cocaine was not due to inhibition of these channels by D2Rs.

Electrogenic coupling with DAT as a reason for lack of L-type function

The DAT affects the reuptake of DA into the presynaptic terminals of DA neurons. In order to do so, it couples two Na^+ ions and one Cl^- ion to each DA molecule, making its activity electrogenic. However, Sonders *et al.* described that the currents mediated by the DAT exceed the predictions from fixed-coupling stoichiometry, turnover rate and transport density (Sonders et al., 1997). Patch experiments have also revealed that transporter channels can have openings and magnitudes of currents that are comparable to ion channels (Wadiche et al., 1995). Carvelli *et al.* described the ability of the DAT to depolarise neurons via a channel mode of conduction in *C. elegans* (Carvelli et al., 2004). In 2015, Cameron *et al.* showed that a human DAT (hDAT) mediated current could induce the activation of L-type VGCCs (Cameron et al., 2015).

The lack of the isradipine effect in the presence of cocaine could potentially be explained by the action of the cocaine itself. By blocking the DAT, the tightly electrically coupled L-type channels were no longer being depolarised and activated therefore removing the effect of isradipine. It can also be hypothesised that since it is the $Ca_v1.3$ subunit containing L-type VGCCs which display a lower voltage activation threshold as well as faster kinetics compared to the $Ca_v1.2$ (Lipscombe *et al.,* 2004), the identity of the L-type VGCCs are $Ca_v1.3$ subunit containing L-type VGCCs.

This hypothesis could be tested by changing the excitability of the membrane by altering extracellular K^+ or Cl^- to change the function of the DAT and test whether the isradipine effect remains occluded. Localization studies would also be useful in

determining whether the DAT and L-type VGCCs are co-localised for the electrogenic activity of the DAT to be effective.

Isradipine is a dihydropyridine and selectively blocks L-type VGCCs, therefore the increase in DA release in the presence of isradipine was surprising. We hypothesised several possible explanations for this effect.

Disinhibition via excess D1 activation

With a D2 antagonist present, the increased extracellular DA caused by the DAT block will result in the downstream activation of D1 receptor. As the most abundant dopamine receptor in the brain, the D1 receptors in the striatum activates cAMP and causes neuronal excitation (Kebabian *et al.,* 1972). In the striatum D1Rs are localised on MSNs as well as interneurons. With D2Rs inhibited, the excess $[DA]_o$ is only activating D1 receptors, we would expect an increased excitability of these cells.

Glutamatergic regulation of DA release has been shown to be mediated by AMPA receptors on MSNs as DA terminals lack ionotropic glutamate receptors. Therefore, it can be hypothesised that excess DA in the presence of the DAT and D2R blocker upregulates glutamatergic regulation of DA release by acting on D1Rs. However, although microdialysis studies have shown a stimulatory effect on striatal DA release, FCV recordings have shown an inhibitory effect. Studies have shown that AMPA receptor activation on MSNs result in H_2O_2 release that leaves the MSN and inhibits DA release from adjacent DA axons. Furthermore, this occurs at a time scale comparable to D2Rs inhibition (500-1000ms). GABAergic MSNs and interneurons form inhibitory synapses with the MSNs as well as modulate DA axons (Gerfen, 1988). Any excess $[DA]_o$ activating D1 receptors would also result in an increase in

GABA release and an inhibition of evoked DA release. Disinhibition via D1Rs is therefore an unlikely explanation for the effect of isradipine.

Loss of inhibitory clamp via action of isradipine

Isradipine belongs to the dihydropyridine family of VGCC blockers. It is a highly selective L-type calcium channels antagonist (Biglan, et al., 2017). Therefore, given its high affinity for L-type VGCC, it is unlikely to have any other effects outside of blocking L-type VGCCs. A possible conjecture for the significant increase in evoked $[DA]_o$ in the presence of cocaine and a D2R blocker is that the isradipine is potentially blocking the release of other inhibitory neurotransmitters in the striatum. While control of DA release is mediated by the firing of DA neurons, local influences have been described to exert their action directly on the terminals of axons. MSNs are the most common type of neurons in the striatum, and are GABAergic neurons (Yager et al., 2015). There are also glutamatergic influences from the prefrontal cortex, amygdala and hippocampus. Furthermore, GABAergic interneurons are also present. These circuits are tightly interconnected. VGCCs play a key role in the release of neurotransmitters and therefore, it is possible that the isradipine is reducing the release of GABA and removing a clamp on DA release, consequently increasing evoked DA release. While L-type VGCCs have been described to contribute to release, traditionally they are not usually involved in vesicle release and are localised post-synaptically (Simms & Zamponi 2014). Furthermore, this effect should have been unmasked with the sole application of isradipine and therefore non-specific effects on other channels is very unlikely.

Ca^{2+} modulated Ca^{2+} inhibition of D2R activity

Dragecevic *et al.,* (2014) described the inhibition of D2R activity by $Ca_v1.3$ VGCCs. They found that D2R desensitization was reduced in post-natal maturation, and that this desensitization required the activity of L-type VGCCs. Upon investigation of this interaction, they found that Ca^{2+} influx via $Ca_v1.3$ channels interacts with the internal sensor NCS-1 to modulate D2R activity. The increase in DA release upon the addition of isradipine could be attributed to the removal of a D2R clamp by L-type inhibition which results in increased DA release. However, the presence of a D2R blocker which should remove any such confounding effect would make this mechanism unlikely.

Effects on δ-receptors and opioid receptors

There are two receptors that could also potentially be underlying the increase. The sigma-1 receptor (Sig-1R) and opioid receptors.

Sig-1Rs are endoplasmic reticular proteins that usually reside specifically between the ER and the mitochondria, but which have been shown to translocate to the plasma membrane and interact with ion channels and receptors. The Sig-1R was originally thought to be a subtype of opioid receptors but were subsequently classified as a separate group (Su et al., 1988). Sig-1Rs promote cell survival by attenuating and modulating Ca^{2+} signalling from the ER. Immunostaining studies have colocalised the D1 receptors and the Sig-1Rs. A functional interaction between the Sig-1Rs and D2R via heteromerization has also been identified. Cocaine is thought to bind to this heteromer and inhibit a D2R activity and the downstream kinase-MAPK pathway (Navarro et al., 2013). Therefore, the increase in DA in the presence of isradipine could be attributed to the removal of D2R inhibition. However,

D2R blockers are also present under control conditions and should account for these effects.

Cocaine is well described as a DAT blocker and is usually associated with altered DA function. However, it has also been shown to have profound effects on the endogenous cannabinoid system. There are two main opioid circuits involved in the dopaminergic system; μ-Opioid receptor (MOP-r) activation results in the release of DA (Zhang et al. 2009, Spanagal *et al.,* 1990) while κ- Opioid receptor (KOP-r) activation decreases extracellular DA levels (Y. Zhang et al., 2004). Chronic cocaine exposure results in increased levels of MOP-r (Unterwald *et al.,* 1992). Therefore, while the exposure to cocaine might not have been chronic, it can be hypothesised that the addition of isradipine resulted in an interaction with the opioid receptors and the increase in DA levels was due to the activation of MOP-r. This hypothesis can be easily tested by using other DAT blockers such as nomifensine and GBR (Yorgason *et al.,* 1988).

There is no clear straightforward explanation for the increase in DA in the presence of isradipine. It is possible a combination of the factors outlined above might be at work. Further experiments to eliminate each explanation will be required.

4.4. D2-autoreceptor function differs between the CPu and the NAc

The activation of D2Rs differs between the CPu and NAc. Under a single stimulation protocol, the activation of D2Rs are greater, although not significantly so, in the CPu. However, the opposite is true at longer stimulation protocols. Under train stimulation protocols, the activation of D2Rs is greater in the NAc than the CPu. It is significantly greater at 5Hz train stimulation. This can be attributed to the activation kinetics of the D2Rs. Phillips *et al.,* (2002) showed that the activation of D2Rs in the CPu and NAc both start 200 msec after initial stimulation but peak activation occurs earlier in the CPu (~550ms) while it occurs slower in the NAc (~700ms).

The CPu and the NAc are functionally different, driving motor control and limbic behaviours respectively. Studies *in vivo* and *in vitro* have shown a gradient from the dorsal to the ventral striatum in the absolute concentration of DA release as well as in reuptake kinetics (Cragg et al., 2002; Jones et al., 1995). Brimblecombe *et al.* (2015) described the VGCCs modulating DA release in the NAc. DA release is modulated only by N- and Q-type channels in the NAc, whereas in the CPu, both L-type and T-type channels were also shown to play a role. Furthermore, L-type and T-type functions could be unmasked in the NAc by increasing levels of extracellular calcium (Brimblecombe *et al.,*2015). The sensitivity of D2Rs and the rate of activation too differ between the two regions. Marcott *et al.* (2018) recently highlighted D2 receptor-mediated synaptic currents from MSNs in the NAc were slower than those from MSNs in the dorsal striatum. Furthermore, they also found that D2 receptors in the NAc via a preferential coupling to $G_{\alpha o}$, are more sensitive to dopamine.

4.4.1. D2Rs inhibit L-type VGCC activity in the NAc

We were able to unmask an L-type VGCC function in the presence of a D2R blocker in the NAc. The sole application of isradipine in the NAc did not modify evoked DA release, however in the presence of a D2 blocker, the addition of isradipine decreased evoked DA release to 80% pre-isradipine levels. In the NAc, the function of L-type VGCCs appears to be under the control of D2 receptors. Understanding and investigating this additional layer of L-type inhibition in the striatum might add to our understanding of the variation in VGCC contribution to dopaminergic neurotoxicity in the case of PD.

The stimulation protocol under which this effect was seen was with a single pulse which indicates that longer stimulation protocols are unnecessary to elicit D2 function. An activation of D2 receptors under basal conditions which allows for the control of L-type VGCCs under these conditions can be hypothesised. Phasic dopamine transients from burst activity are thought to modulate D1Rs while background tonic DA levels from pacemaking have been shown to regulate high-affinity D2 receptors (Keefe et al., 1993; Richfield at al., 1989; Surmeier et al., 2011). There is precedence for D2Rs in the NAc to undergo maximal activation with small concentrations of DA. Marcott *et al.,* (2018) determine that NAc D2Rs on MSNs are saturated by lower DA concentration. If D2Rs on the axons have similar kinetics it is possible that even with a single stimulation, D2Rs are active and inhibiting L-type VGCCs.

However, in these slices, we cannot be sure if the axons are still undergoing phasic and tonic DA release. Furthermore, the pause between two successive action potentials during tonic firing, which allows for the maintenance of the basal $[DA]_{o,}$ exceeds 200 ms, and the DA released by one AP is thought to be cleared before the next (Sulzer *et al.,* 2016). Therefore, the time course over which the inhibition of L-type VGCCs must happen, a mere couple of milliseconds, seems to point to modulation via a βγ- coupled tonic block. It also raises questions regarding the localization of the D2R and the L-type VGCCs which could be potentially answered using immunohistochemistry as well as FRET. A close localization could explain the presence of an inhibition of the L-type VGCC block in the NAc while it is lacking in the CPu.

4.5. Implication for PD and neuropsychiatric disorders

Modulators of DA neurotransmission are commonly used as pharmacological targets for various medical conditions that the dopaminergic system is a part of, including PD, schizophrenia, bipolar disorder and addiction. Both L-type VGCCs and D2Rs are current targets for alleviating the symptoms of many of these disorders.

Implications for PD

The core motor symptoms in PD are caused by the selective loss of SNc dopaminergic neurons. The underlying cause of the high susceptibility of these neurons that results in pathogenesis is still unclear (Surmeier & Sulzer, 2013). Their unique utilisation of Ca^{2+} during dendritic oscillations is thought to result in elevated mitochondrial oxidative stress levels (Guzman et al., 2009). However, studies have shown that pacemaking itself is not Ca^{2+} -dependent. In the absence of L-type VGCCs, Na^{+} and K^{+} channels maintain the pacemaking. In the VTA, Na^{+} drives the

pacemaker activaity (Khaliq & Bean, 2010). SNc neurons have also been shown to recover a Na^+ driven oscillation when L-type VGCCs were inhibited (Chan et al., 2007).

Changes in calcium handling occur in mice with age. Juvenile mice DA neurons in the SNc and VTA use Na^+ currents to drive their SOPs but upon reaching adulthood, SNc neurons switch to a Ca^{2+} driven current while VTA neurons remain using Na^+ (Chan et al., 2007). The interaction between L-type VGCCs and D2Rs in the NAc could be hypothesised to be another mechanism through which L-type activity is supressed in VTA neurons, thus offering neuroprotection.

Studies have shown that the D2R mRNA for both isoforms display a negative correlation with age, with a dramatic decrease in early age groups into adolescence (Rothmond, Weickert, & Webster, 2012). There have been no studies looking at differences in localization in the dorsal and ventral striatum and changes to activity of these receptors over development. However, Dwyer & Leslie (2016) showed that adolescent animals showed enhanced D2-like control over locomotor and repetitive behaviour, setting a precedence for a switch in D2R activity at the point of adolescence.

Investigating D2Rs activity and expression and differences in these across the striatum at the time of the switch in the currents driving the pacemaking might shed light on development differences across the neurons in the SNc and VTA that would result in greater susceptibility of SNc neurons. Understanding differences in the modulation of L-type VGCCs by D2R, would allow us to gain a better understanding of the neuroprotection that this interaction could offer VTA neurons.

Implications for neuropsychiatric conditions and addiction

D2R antagonists are commonly used as antipsychotics to alleviate symptoms of schizophrenia and bipolar disease. In schizophrenia, D2R partial agonists are used to mitigate the effects of an overactive mesolimbic DA pathway and reduce positive symptoms (Stahl & Buckley, 2007). Understanding the implications of the interactions that D2Rs have in controlling DA release via interactions with other modulators such as the L-type VGCC could allow for more effective and early intervention.

The nucleus accumbens is implicated to play an important role in cognitive processing and addiction (Hyman, Malenka, & Nestler, 2006). Striatal long-term depression (LTD) requires the activation of D2Rs which is thought to facilitate the activation of L-type VGCCs by reducing M1 receptor tone (Wang et al., 2006). Chronic application of cocaine has been shown to decrease D2R levels (Thompson, Martini, & Whistler, 2010). The cellular mechanisms that lead to craving-induced cocaine relapses can be hypothesised to be influenced by the interactions between L-type VGCCs and D2Rs. The decrease in D2Rs with chronic cocaine application can be conjectured to lead to decrease in the fine controls balancing LTP and LTD in the striatum.

4.6. Concluding remarks

The striatum is highly heterogenous and there appear to be several layers of modulation of L-type VGCCs across the striatum. Understanding the complex interactions between the various receptors and channels is important for understanding the cause behind debilitating neuromotor and neuropsychiatric conditions. In this thesis, I investigated D2R modulation of L-type VGCCs and their interaction in controlling striatal DA release. I have illustrated D2Rs do not interact with L-type VGCCs in the CPu, however in the NAc, D2Rs inhibit L-type function.

Bibliography

Beaulieu, J.-M., & Gainetdinov, R. R. (2011). The Physiology, Signaling, and Pharmacology of Dopamine Receptors. *Pharmacological Reviews*, *63*(1), 182–217. https://doi.org/10.1124/pr.110.002642

Beckstead, M. J., & Williams, J. T. (2007). Long-term depression of a dopamine IPSC. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *27*(8), 2074–2080. https://doi.org/10.1523/JNEUROSCI.3251-06.2007

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., & Seitelberger, F. (1973). Brain dopamine and the syndromes of Parkinson and Huntington Clinical, morphological and neurochemical correlations. *Journal of the Neurological Sciences*, *20*(4), 415–455. https://doi.org/10.1016/0022-510X(73)90175-5

Bolam, J. P., Wainer, B. H., & Smith, A. D. (1984). Characterization of cholinergic neurons in the rat neostriatum. A combination of choline acetyltransferase immunocytochemistry, Golgi-impregnation and electron microscopy. *Neuroscience*, *12*(3), 711–718. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6382048

Bourinet, E., Soong, T. W., Sutton, K., Slaymaker, S., Mathews, E., Monteil, A., … Snutch, T. P. (1999). Splicing of alpha 1A subunit gene generates phenotypic variants of P- and Q-type calcium channels. *Nature Neuroscience*, *2*(5), 407–415. https://doi.org/10.1038/8070

Brimblecombe, K. R., Gracie, C. J., Platt, N. J., & Cragg, S. J. (2015). Gating of dopamine transmission by calcium and axonal N-, Q-, T- and L-type voltage-gated calcium channels differs between striatal domains. *The Journal of Physiology*, *593*(4), 929–946. https://doi.org/10.1113/jphysiol.2014.285890

Bryant, C. D. (2011). The blessings and curses of C57BL/6 substrains in mouse genetic studies. *Annals of the New York Academy of Sciences*, *1245*(1), 31–33. https://doi.org/10.1111/j.1749-6632.2011.06325.x

Cachope, R., Mateo, Y., Mathur, B. N., Irving, J., Wang, H.-L., Morales, M., … Cheer, J. F. (2012). Selective Activation of Cholinergic Interneurons Enhances Accumbal Phasic Dopamine Release: Setting the Tone for Reward Processing. *Cell Reports*, *2*(1), 33–41. https://doi.org/10.1016/j.celrep.2012.05.011

Cahill, P. S., Walker, Q. D., Finnegan, J. M., Mickelson, G. E., Travis, E. R., & Wightman, R. M. (1996). Microelectrodes for the measurement of catecholamines in biological systems. *Analytical Chemistry*, *68*(18), 3180–3186. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8797378

Calipari, E. S., Huggins, K. N., Mathews, T. A., & Jones, S. R. (2012). Conserved dorsal–ventral gradient of dopamine release and uptake rate in mice, rats and rhesus macaques. *Neurochemistry International*, *61*(7), 986–991. https://doi.org/10.1016/j.neuint.2012.07.008

Cameron, K. N., Solis, E., Ruchala, I., De Felice, L. J., & Eltit, J. M. (2015). Amphetamine activates calcium channels through dopamine transporter-mediated depolarization. *Cell Calcium*, *58*(5), 457–466. https://doi.org/10.1016/J.CECA.2015.06.013

Cardozo, D. L., & Bean, B. P. (1995). Voltage-dependent calcium channels in rat midbrain dopamine neurons: modulation by dopamine and GABAB receptors. *Journal of Neurophysiology*, *74*(3), 1137–1148. https://doi.org/10.1152/jn.1995.74.3.1137

Carvelli, L., McDonald, P. W., Blakely, R. D., & DeFelice, L. J. (2004). Dopamine transporters depolarize neurons by a channel mechanism. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(45), 16046–16051. https://doi.org/10.1073/pnas.0403299101

Cass, W. A., & Gerhardt, G. A. (1994). Direct in vivo evidence that D2 dopamine receptors can modulate dopamine uptake. *Neuroscience Letters*, *176*(2), 259–263. https://doi.org/10.1016/0304-3940(94)90096-5

Castro, S. W., & Strange, P. G. (1993). Coupling of D2 and D3 dopamine receptors to G-proteins. *FEBS Letters*, *315*(3), 223–226. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8422910

Chan, C. S., Guzman, J. N., Ilijic, E., Mercer, J. N., Rick, C., Tkatch, T., … Surmeier, D. J. (2007). 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. *Nature*, *447*(7148), 1081–1086. https://doi.org/10.1038/nature05865

Chang, C.-W., Chiang, C.-W., & Jackson, M. B. (2017). Fusion pores and their control of neurotransmitter and hormone release. *The Journal of General Physiology*, *149*(3), 301–322. https://doi.org/10.1085/jgp.201611724

Chinta, S. J., & Andersen, J. K. (2005). Dopaminergic neurons. *The International Journal of Biochemistry & Cell Biology*, *37*(5), 942–946. https://doi.org/10.1016/j.biocel.2004.09.009

Cho, S., Wood, A., & Bowlby, M. R. (2007). Brain slices as models for neurodegenerative disease and screening platforms to identify novel therapeutics. *Current Neuropharmacology*, *5*(1), 19–33. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18615151

Clapham, D. E. (2007). Calcium Signaling. *Cell*, *131*(6), 1047–1058. https://doi.org/10.1016/j.cell.2007.11.028

Cragg, S. J., Hille, C. J., & Greenfield, S. A. (2002). Functional domains in dorsal striatum of the nonhuman primate are defined by the dynamic behavior of dopamine. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(13), 5705–5712. https://doi.org/20026534

Damier, P., Hirsch, E. C., Agid, Y., & Graybiel, A. M. (1999). The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain : A Journal of Neurology*, *122 (Pt 8)*, 1437–1448. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10430830

Day, M., Wang, Z., Ding, J., An, X., Ingham, C. A., Shering, A. F., … Surmeier, D. J. (2006). Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nature Neuroscience*, *9*(2), 251–259. https://doi.org/10.1038/nn1632

Dickson, D. W., Braak, H., Duda, J. E., Duyckaerts, C., Gasser, T., Halliday, G. M., … Litvan, I. (2009). Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. *The Lancet Neurology*, *8*(12), 1150–1157. https://doi.org/10.1016/S1474-4422(09)70238-8

Ding, J. B., Guzman, J. N., Peterson, J. D., Goldberg, J. A., & Surmeier, D. J. (2010). Thalamic gating of corticostriatal signaling by cholinergic interneurons. *Neuron*, *67*(2), 294–307. https://doi.org/10.1016/j.neuron.2010.06.017

Dolphin, A. C. (2013a). The α2δ subunits of voltage-gated calcium channels. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1828*(7), 1541–1549. https://doi.org/10.1016/J.BBAMEM.2012.11.019

Dolphin, A. C. (2013b). The α2δ subunits of voltage-gated calcium channels. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1828*(7), 1541–1549. https://doi.org/10.1016/j.bbamem.2012.11.019

Dragicevic, E., Poetschke, C., Duda, J., Schlaudraff, F., Lammel, S., Schiemann, J., … Liss, B. (2014). Cav1.3 channels control D2-autoreceptor responses via NCS-1 in substantia nigra dopamine

neurons. *Brain : A Journal of Neurology*, *137*(Pt 8), 2287–2302. https://doi.org/10.1093/brain/awu131

Evans, M. J. (2001). The cultural mouse. *Nature Medicine*, *7*(10), 1081–1083. https://doi.org/10.1038/nm1001-1081

Ewing, A. G., Bigelow, J. C., & Wightman, R. M. (1983). Direct in vivo monitoring of dopamine released from two striatal compartments in the rat. *Science (New York, N.Y.)*, *221*(4606), 169–171. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6857277

Ewing, A. G., Wlghtman, R. M., & Dayton, M. A. (1982). *In Vivo Voltammetry with Electrodes that Discriminate between Dopamine and Ascorbate*. *Brain Research* (Vol. 249). Retrieved from https://ac.els-cdn.com/0006899382900701/1-s2.0-0006899382900701-main.pdf?_tid=32878b49-f72d-42d2-99ad-56a89c2be5cd&acdnat=1535817657_1d9e6b6fdd32e991c9e87136a9cfacfb

Fahn, S. (2003). Description of Parkinson's disease as a clinical syndrome. *Annals of the New York Academy of Sciences*, *991*, 1–14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12846969

Fearnley, J. M., & Lees, A. J. (1991). Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain : A Journal of Neurology*, *114 (Pt 5)*, 2283–2301. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1933245

Ford, C. P. (2014). The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. *Neuroscience*, *282*, 13–22. https://doi.org/10.1016/j.neuroscience.2014.01.025

Fulton, S., Thibault, D., Mendez, J. A., Lahaie, N., Tirotta, E., Borrelli, E., … Trudeau, L.-E. (2011). Contribution of Kv1.2 voltage-gated potassium channel to D2 autoreceptor regulation of axonal dopamine overflow. *The Journal of Biological Chemistry*, *286*(11), 9360–9372. https://doi.org/10.1074/jbc.M110.153262

Gagnon, D., Petryszyn, S., Sanchez, M. G., Bories, C., Beaulieu, J. M., De Koninck, Y., … Parent, M. (2017). Striatal Neurons Expressing D1 and D2 Receptors are Morphologically Distinct and Differently Affected by Dopamine Denervation in Mice. *Scientific Reports*, *7*(1), 41432. https://doi.org/10.1038/srep41432

Galvan, A., & Wichmann, T. (2008). Pathophysiology of Parkinsonism. *Clinical Neurophysiology*, *119*(7), 1459–1474. https://doi.org/10.1016/j.clinph.2008.03.017

Gerfen, C. R. (1988). Synaptic organization of the striatum. *Journal of Electron Microscopy Technique*, *10*(3), 265–281. https://doi.org/10.1002/JEMT.1060100305

Gerfen, C. R., & Bolam, J. P. (2017). The Neuroanatomical Organization of the Basal Ganglia. *Handbook of Behavioral Neuroscience*, *24*, 3–32. https://doi.org/10.1016/B978-0-12-802206-1.00001-5

Grace, A. A., & Bunney, B. S. (1984a). The control of firing pattern in nigral dopamine neurons: burst firing. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *4*(11), 2877–2890. https://doi.org/10.1523/JNEUROSCI.04-11-02877.1984

Grace, A. A., & Bunney, B. S. (1984b). The control of firing pattern in nigral dopamine neurons: single spike firing. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *4*(11), 2866–2876. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6150070

Guzman, J. N., Sánchez-Padilla, J., Chan, C. S., & Surmeier, D. J. (2009). Robust pacemaking in substantia nigra dopaminergic neurons. *The Journal of Neuroscience : The Official Journal of the*

Society for Neuroscience, *29*(35), 11011–11019. https://doi.org/10.1523/JNEUROSCI.2519-09.2009

Haber, S. N., Fudge, J. L., & McFarland, N. R. (2000). Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(6), 2369–2382. https://doi.org/10.1523/JNEUROSCI.20-06-02369.2000

HEBB, C. O., & SILVER, A. (1956). Choline acetylase in the central nervous system of man and some other mammals. *The Journal of Physiology*, *134*(3), 718–728. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/13398956

Herlitze, S., Garcia, D. E., Mackie, K., Hille, B., Scheuer, T., & Catterall, W. A. (1996). Modulation of Ca2+ channels βγ G-protein py subunits. *Nature*, *380*(6571), 258–262. https://doi.org/10.1038/380258a0

Hernandez-Lopez, S., Tkatch, T., Perez-Garci, E., Galarraga, E., Bargas, J., Hamm, H., & Surmeier, D. J. (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+ currents and excitability via a novel PLC[beta]1-IP3-calcineurin-signaling cascade. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(24), 8987–8995. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11124974

Huffman, M. L., & Venton, B. J. (2009). Carbon-fiber microelectrodes for in vivo applications. *The Analyst*, *134*(1), 18–24. https://doi.org/10.1039/b807563h

Hyman, S. E., Malenka, R. C., & Nestler, E. J. (2006). Neural mechanisms of addiction: the role of reward-related learning and memory. *Annual Review of Neuroscience*, *29*(1), 565–598. https://doi.org/10.1146/annurev.neuro.29.051605.113009

Ingram, S. L., Prasad, B. M., & Amara, S. G. (2002). Dopamine transporter–mediated conductances increase excitability of midbrain dopamine neurons. *Nature Neuroscience*, *5*(10), 971–978. https://doi.org/10.1038/nn920

John, C. E., & Jones, S. R. (2007). *Fast Scan Cyclic Voltammetry of Dopamine and Serotonin in Mouse Brain Slices*. *Electrochemical Methods for Neuroscience*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21204393

Jones, S. R., Garris, P. A., & Wightman, R. M. (1995). Different effects of cocaine and nomifensine on dopamine uptake in the caudate-putamen and nucleus accumbens. *Journal of Pharmacology and Experimental Therapeutics*, *274*(1).

Jucker, M. (2010). The benefits and limitations of animal models for translational research in neurodegenerative diseases. *Nature Medicine*, *16*(11), 1210–1214. https://doi.org/10.1038/nm.2224

Katz, B., & Miledi, R. (1968). The effect of local blockage of motor nerve terminals. *The Journal of Physiology*, *199*(3), 729–741. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4303919

Kawagoe, K. T., Zimmerman, J. B., & Wightman, R. M. (1993). Principles of voltammetry and microelectrode surface states. *Journal of Neuroscience Methods*, *48*(3), 225–240. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8412305

Kawaguchi, Y., Aosaki, T., & Kubota, Y. (1997). Cholinergic and GABAergic interneurons in the striatum. *Nihon Shinkei Seishin Yakurigaku Zasshi = Japanese Journal of Psychopharmacology*, *17*(2), 87–90. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9201728

Kebabian, J. W., Petzold, G. L., & Greengard, P. (1972). Dopamine-sensitive adenylate cyclase in

caudate nucleus of rat brain, and its similarity to the "dopamine receptor". *Proceedings of the National Academy of Sciences of the United States of America*, *69*(8), 2145–2149. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4403305

Keefe, K. A., Zigmond, M. J., & Abercrombie, E. D. (1993). In vivo regulation of extracellular dopamine in the neostriatum: influence of impulse activity and local excitatory amino acids. *Journal of Neural Transmission. General Section*, *91*(2–3), 223–240. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8099798

Kemp, J. M., & Powell, T. P. (1971). The structure of the caudate nucleus of the cat: light and electron microscopy. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *262*(845), 383–401. https://doi.org/10.1098/RSTB.1971.0102

Khaliq, Z. M., & Bean, B. P. (2010). Pacemaking in dopaminergic ventral tegmental area neurons: depolarizing drive from background and voltage-dependent sodium conductances. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(21), 7401–7413. https://doi.org/10.1523/JNEUROSCI.0143-10.2010

Kordower, J. H., Olanow, C. W., Dodiya, H. B., Chu, Y., Beach, T. G., Adler, C. H., … Bartus, R. T. (2013). Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. *Brain*, *136*(8), 2419–2431. https://doi.org/10.1093/brain/awt192

Lacey, M. G., Mercuri, N. B., & North, R. A. (1987). Dopamine acts on D2 receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. *The Journal of Physiology*, *392*, 397–416. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2451725

Lipscombe, D., Helton, T. D., & Xu, W. (2004). L-Type Calcium Channels: The Low Down. *Journal of Neurophysiology*, *92*(5), 2633–2641. https://doi.org/10.1152/jn.00486.2004

Macpherson, T., Morita, M., & Hikida, T. (2014). Striatal direct and indirect pathways control decision-making behavior. *Frontiers in Psychology*, *5*, 1301. https://doi.org/10.3389/fpsyg.2014.01301

Mazzoni, P., Shabbott, B., & Cortés, J. C. (2012). Motor control abnormalities in Parkinson's disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(6), a009282. https://doi.org/10.1101/cshperspect.a009282

Melikian, H. E., & Buckley, K. M. (1999). Membrane trafficking regulates the activity of the human dopamine transporter. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *19*(18), 7699–7710. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10479674

Missale, C., Castelletti, L., Govoni, S., Spano, P. F., Trabucchi, M., & Hanbauer, I. (1985). Dopamine uptake is differentially regulated in rat striatum and nucleus accumbens. *Journal of Neurochemistry*, *45*(1), 51–56. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3998732

Molochnikov, I., & Cohen, D. (2014). Hemispheric differences in the mesostriatal dopaminergic system. *Frontiers in Systems Neuroscience*, *8*, 110. https://doi.org/10.3389/fnsys.2014.00110

Montmayeur, J. P., Guiramand, J., & Borrelli, E. (1993). Preferential coupling between dopamine D2 receptors and G-proteins. *Molecular Endocrinology (Baltimore, Md.)*, *7*(2), 161–170. https://doi.org/10.1210/mend.7.2.7682286

Morales, M., & Margolis, E. B. (2017). Ventral tegmental area: cellular heterogeneity, connectivity and behaviour. *Nature Reviews Neuroscience*, *18*(2), 73–85. https://doi.org/10.1038/nrn.2016.165

Moran, C. J., Ramesh, A., Brama, P. A. J., O'Byrne, J. M., O'Brien, F. J., & Levingstone, T. J. (2016). The benefits and limitations of animal models for translational research in cartilage repair. *Journal of Experimental Orthopaedics*, *3*(1), 1. https://doi.org/10.1186/s40634-015-0037-x

Navarro, G., Moreno, E., Bonaventura, J., Brugarolas, M., Farré, D., Aguinaga, D., … McCormick, P. J. (2013). Cocaine Inhibits Dopamine D2 Receptor Signaling via Sigma-1-D2 Receptor Heteromers. *PLoS ONE*, *8*(4), e61245. https://doi.org/10.1371/journal.pone.0061245

Neve, K. A., Seamans, J. K., & Trantham-Davidson, H. (2004). Dopamine receptor signaling. *Journal of Receptor and Signal Transduction Research*, *24*(3), 165–205. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15521361

Nicola, S. M., Surmeier, D. J., & Malenka, R. C. (2000). Dopaminergic Modulation of Neuronal Excitability in the Striatum and Nucleus Accumbens. *Annual Review of Neuroscience*, *23*(1), 185–215. https://doi.org/10.1146/annurev.neuro.23.1.185

O'Neill, B., Patel, J. C., & Rice, M. E. (2017). Characterization of Optically and Electrically Evoked Dopamine Release in Striatal Slices from Digenic Knock-in Mice with DAT-Driven Expression of Channelrhodopsin. *ACS Chemical Neuroscience*, *8*(2), 310–319. https://doi.org/10.1021/acschemneuro.6b00300

Palij, P., Bull, D. R., Sheehan, M. J., Millar, J., Stamford, J., Kruk, Z. L., & Humphrey, P. P. A. (1990). Presynaptic regulation of dopamine release in corpus striatum monitored in vitro in real time by fast cyclic voltammetry. *Brain Research*, *509*(1), 172–174. https://doi.org/10.1016/0006-8993(90)90329-A

Patel, J. C., & Rice, M. E. (2013). Monitoring Axonal and Somatodendritic Dopamine Release Using Fast-Scan Cyclic Voltammetry in Brain Slices. In *Methods in molecular biology (Clifton, N.J.)* (Vol. 964, pp. 243–273). https://doi.org/10.1007/978-1-62703-251-3_15

Perez-Reyes, E., Cribbs, L. L., Daud, A., Lacerda, A. E., Barclay, J., Williamson, M. P., … Lee, J.-H. (1998). Molecular characterization of a neuronal low-voltage-activated T-type calcium channel. *Nature*, *391*(6670), 896–900. https://doi.org/10.1038/36110

Phillips, P. E. M., Hancock, P. J., & Stamford, J. A. (2002). Time window of autoreceptor-mediated inhibition of limbic and striatal dopamine release. *Synapse*, *44*(1), 15–22. https://doi.org/10.1002/syn.10049

Ping, H. X., & Shepard, P. D. (1999). Blockade of SK-Type Ca 2+ -Activated K+ Channels Uncovers a Ca 2+-Dependent Slow Afterdepolarization in Nigral Dopamine Neurons. *Journal of Neurophysiology*, *81*(3), 977–984. https://doi.org/10.1152/jn.1999.81.3.977

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., … Nussbaum, R. L. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science (New York, N.Y.)*, *276*(5321), 2045–2047. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9197268

Pothos, E. N., Przedborski, S., Davila, V., Schmitz, Y., & Sulzer, D. (1998). D2-Like dopamine autoreceptor activation reduces quantal size in PC12 cells. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *18*(15), 5575–5585. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9671649

Pramod, A. B., Foster, J., Carvelli, L., & Henry, L. K. (2013). SLC6 transporters: structure, function, regulation, disease association and therapeutics. *Molecular Aspects of Medicine*, *34*(2–3), 197–219. https://doi.org/10.1016/j.mam.2012.07.002

Purves, D., Augustine, G. J., Fitzpatrick, D., Katz, L. C., LaMantia, A.-S., McNamara, J. O., & Williams, S.

M. (2001). Circuits within the Basal Ganglia System. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK10847/

Ramamoorthy, S., Shippenberg, T. S., & Jayanthi, L. D. (2011). Regulation of monoamine transporters: Role of transporter phosphorylation. *Pharmacology & Therapeutics*, *129*(2), 220–238. https://doi.org/10.1016/J.PHARMTHERA.2010.09.009

Rice, M. E., & Cragg, S. J. (2004). Nicotine amplifies reward-related dopamine signals in striatum. *Nature Neuroscience*, *7*(6), 583–584. https://doi.org/10.1038/nn1244

Rice, M. E., Patel, J. C., & Cragg, S. J. (2011). Dopamine release in the basal ganglia. *Neuroscience*, *198*, 112–137. https://doi.org/10.1016/j.neuroscience.2011.08.066

Richfield, E. K., Penney, J. B., & Young, A. B. (1989). Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. *Neuroscience*, *30*(3), 767–777. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2528080

Ring, H. A., & Serra-Mestres, J. (2002). Neuropsychiatry of the basal ganglia. *Journal of Neurology, Neurosurgery, and Psychiatry*, *72*(1), 12–21. https://doi.org/10.1136/JNNP.72.1.12

Rizo, J., & Xu, J. (2015). The Synaptic Vesicle Release Machinery. *Annual Review of Biophysics*, *44*(1), 339–367. https://doi.org/10.1146/annurev-biophys-060414-034057

Robinson, D. L., Venton, B. J., Heien, M. L. A. V, & Wightman, R. M. (2003). Detecting subsecond dopamine release with fast-scan cyclic voltammetry in vivo. *Clinical Chemistry*, *49*(10), 1763–1773. https://doi.org/10.1373/49.10.1763

Romanelli, R. J., Williams, J. T., & Neve, K. A. (2010). Dopamine Receptor Signaling: Intracellular Pathways to Behavior. In *The Dopamine Receptors* (pp. 137–173). Totowa, NJ: Humana Press. https://doi.org/10.1007/978-1-60327-333-6_6

Rothmond, D. A., Weickert, C. S., & Webster, M. J. (2012). Developmental changes in human dopamine neurotransmission: cortical receptors and terminators. *BMC Neuroscience*, *13*(1), 18. https://doi.org/10.1186/1471-2202-13-18

Schapira, A. H. V. (2006). Etiology of Parkinson's disease. *Neurology*, *66*(10 Suppl 4), S10-23. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16717248

Schultz, W. (2000). Multiple reward signals in the brain. *Nature Reviews Neuroscience*, *1*(3), 199–207. https://doi.org/10.1038/35044563

Sehgal, P. B., & Lee, J. E. (2011). Protein Trafficking Dysfunctions: Role in the Pathogenesis of Pulmonary Arterial Hypertension. *Pulmonary Circulation*, *1*(1), 17–32. https://doi.org/10.4103/2045-8932.78097

Sheng, Z.-H., Rettig, J., Cook, T., & Catterall, W. A. (1996). Calcium-dependent interaction of N-type calcium channels with the synaptic core complex. *Nature*, *379*(6564), 451–454. https://doi.org/10.1038/379451a0

Silberberg, G., & Bolam, J. P. (2015). Local and afferent synaptic pathways in the striatal microcircuitry. *Current Opinion in Neurobiology*, *33*, 182–187. https://doi.org/10.1016/j.conb.2015.05.002

Simms, B. A., & Zamponi, G. W. (2014). Review Neuronal Voltage-Gated Calcium Channels: Structure, Function, and Dysfunction. *NEURON*, *82*, 24–45. https://doi.org/10.1016/j.neuron.2014.03.016

Sonders, M. S., & Amara, S. G. (1996). Channels in transporters. *Current Opinion in Neurobiology*, *6*(3), 294–302. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8794089

Sonders, M. S., Zhu, S. J., Zahniser, N. R., Kavanaugh, M. P., & Amara, S. G. (1997). Multiple ionic conductances of the human dopamine transporter: the actions of dopamine and psychostimulants. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *17*(3), 960–974. https://doi.org/10.1523/JNEUROSCI.17-03-00960.1997

Soong, T. W., Stea, A., Hodson, C. D., Dubel, S. J., Vincent, S. R., & Snutch, T. P. (1993). Structure and functional expression of a member of the low voltage-activated calcium channel family. *Science (New York, N.Y.)*, *260*(5111), 1133–1136. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8388125

Stahl, S. M., & Buckley, P. F. (2007). Negative symptoms of schizophrenia: a problem that will not go away. *Acta Psychiatrica Scandinavica*, *115*(1), 4–11. https://doi.org/10.1111/j.1600-0447.2006.00947.x

Stocco, A., Lebiere, C., & Anderson, J. R. (2010). Conditional routing of information to the cortex: a model of the basal ganglia's role in cognitive coordination. *Psychological Review*, *117*(2), 541–574. https://doi.org/10.1037/a0019077

Striessnig, J. (2013). Lonely but diverse: Cav1.3 L-type Ca2+ channels in cochlear inner hair cells. *Channels*, *7*(3), 133–134. https://doi.org/10.4161/chan.24457

Su, T. P., London, E. D., & Jaffe, J. H. (1988). Steroid binding at sigma receptors suggests a link between endocrine, nervous, and immune systems. *Science (New York, N.Y.)*, *240*(4849), 219–221. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2832949

Südhof, T. C. (2012). Calcium control of neurotransmitter release. *Cold Spring Harbor Perspectives in Biology*, *4*(1), a011353. https://doi.org/10.1101/cshperspect.a011353

Sulzer, D., Cragg, S. J., & Rice, M. E. (2016). Striatal dopamine neurotransmission: Regulation of release and uptake. *Basal Ganglia*, *6*(3), 123–148. https://doi.org/10.1016/J.BAGA.2016.02.001

Surmeier, D. J., Carrillo-Reid, L., & Bargas, J. (2011). Dopaminergic modulation of striatal neurons, circuits, and assemblies. *Neuroscience*, *198*, 3–18. https://doi.org/10.1016/j.neuroscience.2011.08.051

Surmeier, D. J., Schumacker, P. T., Guzman, J. D., Ilijic, E., Yang, B., & Zampese, E. (2017). Calcium and Parkinson's disease. *Biochemical and Biophysical Research Communications*, *483*(4), 1013–1019. https://doi.org/10.1016/j.bbrc.2016.08.168

Surmeier, D. J., Shen, W., Day, M., Gertler, T., Chan, S., Tian, X., & Plotkin, J. L. (2010). The role of dopamine in modulating the structure and function of striatal circuits. *Progress in Brain Research*, *183*, 149–167. https://doi.org/10.1016/S0079-6123(10)83008-0

Surmeier, D. J., & Sulzer, D. (2013). The pathology roadmap in Parkinson disease. *Prion*, *7*(1), 85–91. https://doi.org/10.4161/pri.23582

Takahashi, M., Seagar, M. J., Jones, J. F., Reber, B. F., & Catterall, W. A. (1987). Subunit structure of dihydropyridine-sensitive calcium channels from skeletal muscle. *Proceedings of the National Academy of Sciences of the United States of America*, *84*(15), 5478–5482. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2440051

Takahashi, T., & Momiyama, A. (1993). Different types of calcium channels mediate central synaptic transmission. *Nature*, *366*(6451), 156–158. https://doi.org/10.1038/366156a0

Tepper, J. M., Abercrombie, E. D., & Bolam, J. P. (2007). Basal ganglia macrocircuits. *Progress in Brain Research*, *160*, 3–7. https://doi.org/10.1016/S0079-6123(06)60001-0

Thompson, D., Martini, L., & Whistler, J. L. (2010). Altered Ratio of D1 and D2 Dopamine Receptors in

Mouse Striatum Is Associated with Behavioral Sensitization to Cocaine. *PLoS ONE*, *5*(6), e11038. https://doi.org/10.1371/journal.pone.0011038

Threlfell, S., Lalic, T., Platt, N. J., Jennings, K. A., Deisseroth, K., & Cragg, S. J. (2012). Striatal Dopamine Release Is Triggered by Synchronized Activity in Cholinergic Interneurons. *Neuron*, *75*(1), 58–64. https://doi.org/10.1016/j.neuron.2012.04.038

Trout, S. J., & Kruk, Z. L. (1992). Differences in evoked dopamine efflux in rat caudate putamen, nucleus accumbens and tuberculum olfactorium in the absence of uptake inhibition: influence of autoreceptors. *British Journal of Pharmacology*, *106*(2), 452–458. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1393270

Truong, J. G., Newman, A. H., Hanson, G. R., & Fleckenstein, A. E. (2004). Dopamine D2 receptor activation increases vesicular dopamine uptake and redistributes vesicular monoamine transporter-2 protein. *European Journal of Pharmacology*, *504*(1–2), 27–32. https://doi.org/10.1016/J.EJPHAR.2004.09.049

Unterwald, E. M., Horne-King, J., & Kreek, M. J. (1992). Chronic cocaine alters brain mu opioid receptors. *Brain Research*, *584*(1–2), 314–318. https://doi.org/10.1016/0006-8993(92)90912-S

Venton, B. J., Seipel, A. T., Phillips, P. E. M., Wetsel, W. C., Gitler, D., Greengard, P., … Wightman, R. M. (2006). Cocaine Increases Dopamine Release by Mobilization of a Synapsin-Dependent Reserve Pool. *Journal of Neuroscience*, *26*(12), 3206–3209. https://doi.org/10.1523/JNEUROSCI.4901-04.2006

Venton, B. J., Zhang, H., Garris, P. A., Phillips, P. E. M., Sulzer, D., & Wightman, R. M. (2003). Real-time decoding of dopamine concentration changes in the caudate-putamen during tonic and phasic firing. *Journal of Neurochemistry*, *87*(5), 1284–1295. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14622108

Wadiche, J. I., Amara, S. G., & Kavanaugh, M. P. (1995). *Ion Fluxes Associated with Excitatory Amino Acid Transport*. *Neuron* (Vol. 15). Retrieved from https://www.cell.com/neuron/pdf/0896-6273(95)90159-0.pdf?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2F0896627395901590%3Fshowall%3Dtrue

Wallace, D. C. (2005). A Mitochondrial Paradigm of Metabolic and Degenerative Diseases, Aging, and Cancer: A Dawn for Evolutionary Medicine. *Annual Review of Genetics*, *39*(1), 359–407. https://doi.org/10.1146/annurev.genet.39.110304.095751

Wang, Z., Kai, L., Day, M., Ronesi, J., Yin, H. H., Ding, J., … Surmeier, D. J. (2006). Dopaminergic Control of Corticostriatal Long-Term Synaptic Depression in Medium Spiny Neurons Is Mediated by Cholinergic Interneurons. *Neuron*, *50*(3), 443–452. https://doi.org/10.1016/j.neuron.2006.04.010

Weiss, N., Hameed, S., Fernández-Fernández, J. M., Fablet, K., Karmazinova, M., Poillot, C., … De Waard, M. (2012). A Ca(v)3.2/syntaxin-1A signaling complex controls T-type channel activity and low-threshold exocytosis. *The Journal of Biological Chemistry*, *287*(4), 2810–2818. https://doi.org/10.1074/jbc.M111.290882

Wilson, C. J., & Callaway, J. C. (2000). Coupled Oscillator Model of the Dopaminergic Neuron of the Substantia Nigra. *Journal of Neurophysiology*, *83*(5), 3084–3100. https://doi.org/10.1152/jn.2000.83.5.3084

Woolf, N. J., Butcher, L. L., & BUTCHER Cholinergic, L. L. (1981). *Cholinergic Neurons in the Caudate-stamen Complex Proper Are Intrinsically Organized: A Combined Evans Blue and*

Acetylcholinesterase Analysis. *Brain Research Buttetin* (Vol. 7). Retrieved from https://ac.els-cdn.com/0361923081900046/1-s2.0-0361923081900046-main.pdf?_tid=c3d1f91d-846e-4bda-b1bf-8b76329fcbbf&acdnat=1535971911_709b26c4f1a940461739999ba3fb0b75

Yager, L. M., Garcia, A. F., Wunsch, A. M., & Ferguson, S. M. (2015). NEUROSCIENCE FOREFRONT REVIEW THE INS AND OUTS OF THE STRIATUM: ROLE IN DRUG ADDICTION. https://doi.org/10.1016/j.neuroscience.2015.06.033

Yamada, T., McGeer, P. L., Baimbridge, K. G., & McGeer, E. G. (1990). Relative sparing in Parkinson's disease of substantia nigra dopamine neurons containing calbindin-D28K. *Brain Research*, *526*(2), 303–307. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2257487

Yorgason, J. T., Jones, S. R., & España, R. A. (1988). Low and high affinity dopamine transporter inhibitors block dopamine uptake within 5 sec of intravenous injection. *Neuroscience*, *182*, 125–132. https://doi.org/10.1016/j.neuroscience.2011.03.017

Zaja-Milatovic, S., Milatovic, D., Schantz, A. M., Zhang, J., Montine, K. S., Samii, A., … Montine, T. J. (2005). Dendritic degeneration in neostriatal medium spiny neurons in Parkinson disease. *Neurology*, *64*(3), 545–547. https://doi.org/10.1212/01.WNL.0000150591.33787.A4

Zamponi, G. W., & Currie, K. P. M. (2013). Regulation of CaV2 calcium channels by G protein coupled receptors. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1828*(7), 1629–1643. https://doi.org/10.1016/j.bbamem.2012.10.004

Zhang, H., Maximov, A., Fu, Y., Xu, F., Tang, T.-S., Tkatch, T., … Bezprozvanny, I. (2005). Association of CaV1.3 L-Type Calcium Channels with Shank. *Journal of Neuroscience*, *25*(5), 1037–1049. https://doi.org/10.1523/JNEUROSCI.4554-04.2005

Zhang, Y., Butelman, E. R., Schlussman, S. D., Ho, A., & Kreek, M. J. (2004). Effect of the endogenous ? opioid agonist dynorphin A(1?17) on cocaine-evoked increases in striatal dopamine levels and cocaine-induced place preference in C57BL/6J mice. *Psychopharmacology*, *172*(4), 422–429. https://doi.org/10.1007/s00213-003-1688-3

Zhang, Y., Picetti, R., Butelman, E. R., Schlussman, S. D., Ho, A., & Kreek, M. J. (2009). Behavioral and Neurochemical Changes Induced by Oxycodone Differ Between Adolescent and Adult Mice. *Neuropsychopharmacology*, *34*(4), 912–922. https://doi.org/10.1038/npp.2008.134