Investigating the presynaptic control of striatal dopamine release

DPhil in Physiology, Anatomy and Genetics

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Abstract

Dopamine (DA) is a key neuromodulator in the striatum, and is important for action selection and reinforcement learning. Dysfunctions in striatal DA signalling contribute to numerous disorders including Parkinson's disease (PD) and drug addiction.

Midbrain DA neurons switch from low to high frequency firing in response to reward-related events, which is proposed to increase striatal DA release. However, in addition to DA neuron firing pattern, striatal DA signalling depends upon the short-term plasticity of DA release, which is controlled by presynaptic and local network factors.

This thesis uses fast-scan cyclic voltammetry, in murine striatal slices, to detect subsecond changes in extracellular DA, and investigate the presynaptic control of striatal DA release and release plasticity. Acetylcholine from striatal cholinergic interneurons, acting at nicotinic receptors (nAChRs) on DA terminals, is one factor that strongly influences DA release. This thesis particularly explores how presynaptic factors interact with nAChRs to control DA release.

Firstly, release probability, a key determinant of release plasticity at many synapses, was found to be only weakly related to DA release plasticity, and only when nAChRs are inactive. Secondly, a direct role of the DA uptake transporter (DAT) in controlling DA release plasticity was identified, when nAChRs are inactive. Thirdly, regional differences were identified in the role of the DAT in controlling DA release via control of D_2 receptor activation, when nAChRs are active. Finally, mutant α-synuclein, which causes PD in humans, was found to only subtly affect striatal DA release.

These data suggest that the control of striatal DA release differs substantially from other central transmitters. Release probability and α-synuclein play only minor roles, but nAChRs and the DAT significantly control DA release plasticity. These findings review our understanding of striatal DA release and may have implications for understanding the actions of drugs of abuse and early PD pathogenesis.

Contents

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I recently re-watched the film Chariots of Fire in which Eric Liddell famously says 'I believe God made me for a purpose, but he also made me fast. And when I run I feel His pleasure'. I strongly believe God has given me my love of science, and I have certainly felt God's pleasure while studying for my DPhil. I believe that it is a huge privilege to study God's creation in the way I have been able to over the last three years and I hope and pray that this thesis glorifies Him.

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Abbreviations

6-OHDA	6-hydroxydopamine
β2* nAChR	β2-subunit containing nicotinic acetylcholine receptor
AAV	adeno-associated virus
ACh	acetylcholine
AChE	acetylcholinesterase
aCSF	artificial cerebrospinal fluid
ADHD	attention deficit hyperactivity disorder
AP	anterior-posterior
ATP	adenosine triphosphate
α-syn	α-synuclein
$[Ca^{2+}]_o$	extracellular calcium concentration
CFMs	carbon fibre microelectrodes
ChAT	choline acetyltransferase
ChIs	cholinergic interneurons
ChR2	channelrhodopsin 2
$[Cl^-]_o$	extracellular chloride concentration
CPu	caudate-putamen
$D_{1\text{-}5}Rs$	dopamine (1-5) receptors
D_2L/D_2S	long/short splice variants of dopamine 2 receptor
D-AP5	D-(2*R*)-amino-5-phosphonopentanoate
DA	dopamine
$[DA]_o$	extracellular evoked dopamine concentration
DAT	dopamine uptake transporter
DHβE	dihydro-β-erythroidine
dlCPu	dorsolateral caudate-putamen
DMSO	dimethyl sulphoxide
DV	dorsal-ventral
E1	1^{st} stimulation epoch
E2	2^{nd} stimulation epoch
eYFP	enhanced yellow fluorescent protein
FCV	fast-scan cyclic voltammetry
GPe	globus pallidus external segment
GPi	globus pallidus internal segment
GYKI25466	4-(8-methyl-9*H*-1,3-dioxolo[4,5-*h*][2,3]benzodiazepin-5-yl)-benzenamine hydrochloride
I_h	hyperpolarisation activated cation conductance
IPI	interpulse interval
IPSPs	inhibitory postsynaptic potentials
$[K^+]_o$	extracellular potassium concentration
LED	light emitting diode
LTD	long term depression
LTP	long term potentiation
$M_{1\text{-}5}Rs$	muscarinic (1-5) receptors
mAChRs	muscarinic acetylcholine receptors
MCPG	(S)-α-methyl-4-carboxyphenylglycine
mGluRs	metabotropic glutamate receptors
ML	medial-lateral

(d/i)MSNs	(direct /indirect pathway) medium spiny neurons
NAc	nucleus accumbens
NAcC	nucleus accumbens core
nAChRs	nicotinc acetylcholine receptors
NET	noradrenaline uptake transporter
NMDARs	*N*-methyl-D-aspartate receptor
P2/P1	paired pulse ratio
PBS	phosphate buffered saline
PBS-Tx	phosphate buffered saline containing 0.5% Triton X-100
PET	positron emission tomography
PD	Parkinson's disease
P_r	initial release probability
PrP	mouse prion protein
S3KO	synapsin III knockout mice
SEM	standard error of the mean
SERT	serotonin uptake transporter
SNARE	soluble NSF attachment protein receptor
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
STN	subthalamic nucleus
TANs	tonically active neurons
TH	tyrosine hydroxylase
VGCC	voltage-gated calcium channel
VGSC	voltage-gated sodium channel
VTA	ventral tegmental area
WT	wild-type

1. Introduction

Dopamine (DA) in the striatum plays important roles in locomotion, goal-directed actions and reward-related learning. Dysfunctions in striatal DA are thought to underlie or contribute to many neurological and psychological disorders, including Parkinson's disease (PD) and drug addiction. In order to identify new drug targets or therapeutic strategies for such disorders, it is important to understand how striatal DA release is physiologically controlled, and how it is disrupted in disease. This thesis explores the physiological and pathophysiological control of striatal DA release, particularly the roles and interactions of initial release probability (P_r), presynaptic nicotinic acetylcholine receptors (nAChRs), the dopamine uptake transporter (DAT) and mutant α-synuclein (α-syn) in the control of DA release. These findings may have implications for the actions of drugs of abuse and for understanding early pathological changes in PD.

This chapter will introduce topics which are of relevance to this thesis as a whole. Firstly, this chapter will outline the function and organisation of the striatum and its dopaminergic innervation. Secondly, it will discuss our current understanding of the presynaptic control of DA release and release plasticity. Finally, it will outline the involvement of striatal DA in disease, particularly in PD and drug addiction.

1.1 The striatum and the basal ganglia

1.1.1 Overview

The basal ganglia are a group of interconnected subcortical nuclei which play important roles in action selection, reinforcement learning and decision making (Graybiel *et al.* 1994; Packard and Knowlton 2002; Yin and Knowlton 2006; Balleine *et al.* 2007). The basal ganglia consist of the striatum, the globus pallidus external segment (GPe), the subthalamic nucleus (STN), the globus pallidus internal segment (GPi) and the substantia nigra pars reticulata (SNr). The striatum is the major input nucleus to the basal ganglia and receives glutamatergic afferents from the cortex

and thalamus (Bolam *et al.* 2000). The striatum is composed primarily of GABAergic projection neurons called medium spiny neurons (MSNs)(Alexander and Crutcher 1990). These can be divided into two major populations which, according to a classic model, initiate two pathways through the basal ganglia: the direct and the indirect pathway (Albin *et al.* 1989; Alexander and Crutcher 1990; DeLong 1990; Gerfen *et al.* 1990; Smith *et al.* 1998). Direct pathway MSNs (dMSNs) project directly to the GPi and SNr, which form the output nuclei of the basal ganglia. Indirect pathway MSNs (iMSNs) project to the GPe, which in turn projects to the STN, which then projects to the output nuclei. The output nuclei then send projections back to the thalamus, and to brainstem structures (Albin *et al.* 1989; Alexander and Crutcher 1990; Smith *et al.* 1998). This model is diagrammed in **Fig. 1.1**. All connections within these basal ganglia pathways, with the exception of the STN to output nuclei connection, are GABAergic and hence inhibitory. Therefore, the direct pathway inhibits the output nuclei, resulting in disinhibition of the thalamus, promoting thalamic and cortical activity, while the indirect pathway results in activation of the output nuclei, increasing inhibition of the thalamus and cortex (Alexander and Crutcher 1990; Smith *et al.* 1998; Bolam *et al.* 2000). Hence, the output of the basal ganglia depends upon the balance of activity in the direct and indirect pathways (Albin *et al.* 1989; DeLong 1990; Gerfen *et al.* 1990; Smith *et al.* 1998).

However, this is a highly simplified model and numerous substantial connections outside of this model have been identified. These include collateral projections of dMSNs to the GPe (Kawaguchi *et al.* 1990; Wu *et al.* 2000), a projection from the GPe back to the striatum from a subpopulation of GPe neurons (Staines *et al.* 1981; Rajakumar *et al.* 1994; Bevan *et al.* 1998; Mallet *et al.* 2012), a projection from the GPe directly to the output nuclei of the basal ganglia (Kincaid *et al.* 1991; Bolam and Smith 1992), and the existence of cortical inputs to the STN forming a 'hyperdirect pathway' (Monakow *et al.* 1978; Kitai and Deniau 1981; Bevan *et al.* 1995). This more complex basal ganglia circuitry is shown in **Fig 1.2**. In addition, the simple model of basal ganglia function largely assumes that information is carried by the average firing rate of

neurons in the direct and indirect pathways (Albin *et al.* 1989). However, more recent work has demonstrated that patterns of neuronal firing and coupling of firing patterns between nuclei , as well as firing rate, may be significant in basal ganglia function (Bevan *et al.* 2002). Despite this considerable complexity, the direct/indirect pathway model can still be a useful model for thinking about basal ganglia, and particularly striatal, function.

The basal ganglia show a topographic organisation of function, defined by the topographic nature of striatal inputs (Alexander *et al.* 1986; Haber 2003). The dorsolateral striatum receives inputs largely from sensorimotor areas of the cortex and thalamus, and hence is thought to be involved primarily in action selection and motor learning. Dorsomedial striatal regions receive inputs from associative regions such as the dorsolateral prefrontal cortex and central medial thalamus. The ventral striatum, or nucleus accumbens (NAc), receives inputs from limbic-associated regions including the orbital frontal cortex, anterior cingulate cortex, amygdala, and midline and medial intralaminar nuclei of the thalamus, and hence subserves limbic and reward-associated functions. In turn, these striatal territories project topographically to downstream nuclei of the basal ganglia, and this organisation is maintained throughout outputs to the thalamus and back to cortex. Therefore, there are a number of distinct parallel loops through the basal ganglia with distinct functions (Alexander *et al.* 1991; Haber 2003). However, these loops are not entirely segregated; there are several points of cross-talk between them. For example, there is some overlap in striatal innervation by different cortical regions (Zheng and Wilson 2002; Haber *et al.* 2006), and projections from distinct territories in upstream basal ganglia nuclei show some convergence in downstream structures (Percheron and Filion 1991; Bevan *et al.* 1997). Furthermore, lateral connections within basal ganglia nuclei (Tepper *et al.* 2004), and non-reciprocal feedback connections between nuclei provide further substrates for interaction between functional basal ganglia loops (Joel and Weiner 1997; Haber 2000). Therefore, whilst basal ganglia functions are largely segregated by topography, cross-talk between these parallel

loops allows the basal ganglia to play complex roles which integrate information across these functional divisions, such as the learning of complex behaviours (Haber et al., 2006).

In summary the basal ganglia, and particularly the striatum, which forms the major input nucleus to the basal ganglia and where information processing is best understood, are key structures subserving complex functions, particularly involving decision making and the learning of complex behaviours (Graybiel et al., 1994, Packard and Knowlton, 2002, Yin and Knowlton, 2006, Balleine et al., 2007). Different striatal regions have distinct functions, but interactions between these regions are significant for complex behaviours.

1.1.2 Striatal organisation

MSNs comprise over 90% of striatal neurons and are the major projection neurons of the striatum (Kemp and Powell 1971; Graveland and DiFiglia 1985). A diverse collection of interneurons makes up the remaining 5-10% of striatal neurons, or perhaps slightly more in primates or humans (Graveland and DiFiglia 1985; Graveland *et al.* 1985). Around 0.5-3% of striatal neurons are large apsiny cholinergic interneurons (ChIs) (Kemp and Powell 1971; Bolam *et al.* 1984; Graveland and DiFiglia 1985), which release acetylcholine (ACh). The importance of striatal ChIs is discussed further below. The remaining striatal neurons are GABAergic interneurons which can be divided into several classes: parvalbumin-positive fast-spiking interneurons, somatostatin, neuropeptide Y/nitric oxide synthase-positive interneurons, calretinin-positive interneurons, and potentially more (Tepper *et al.* 2010). The complete roles of these GABAergic interneurons are unknown, but they are thought to contribute to local striatal circuitry, forming a complex microcircuit of feedforward and feedback loops, and likely play key roles in information processing in the striatum (Kawaguchi et al., 1995, Tepper et al., 2010).

1.1.3 DA innervation of the striatum

In addition to cortical and thalamic inputs, the striatum receives a dense dopaminergic innervation from the midbrain. There are two main populations of midbrain DA neurons which innervate the striatum: one in the substantia nigra pars compacta (SNc), which can be divided into a dorsal and ventral tier, and the other in the ventral tegmental area (VTA). These DA neuron populations differ in a number of characteristics, such as their pattern of development (Gerfen *et al.* 1987), and expression and function of many proteins, including the Ca^{2+} binding protein calbindin, expressed by neurons in the VTA and dorsal tier SNc but not ventral SNc (Gerfen *et al.* 1987), the DAT, expressed at lower levels in VTA than SNc (Blanchard *et al.* 1994), and Ca^{2+} and K^{+} channels, which have different functions in the two populations (Liss *et al.* 2005; Chan *et al.* 2007). As described above for glutamatergic inputs, DA neurons also project topographically to the striatum. The lateral and ventral SNc project largely to the dorsal striatum, whereas the medial and dorsal SNc and VTA project to the more limbic-associated ventral striatum (Haber 2000). Additionally, the striatum can be divided into compartments called the patches and matrix, based on development pattern and expression of several biochemical markers (Gerfen 1992). DA neuron populations also project differentially to these compartments, with ventral SNc neurons preferentially projecting to patches and VTA and dorsal SNc neurons preferentially projecting to the matrix compartment (Gerfen *et al.* 1987).

1.1.3.1 DA in the striatum

The striatal DA innervation is incredibly dense with DA axons forming large, dense arborisations. Indeed, levels of dopaminergic markers in the striatum, such as tyrosine hydroxylase (TH), the rate-limiting enzyme involved in DA synthesis, and the DAT, are among the highest of any brain region (Pickel *et al.* 1975; Ciliax *et al.* 1995), and single DA neurons have been reported to arborise throughout up to 6% of the volume of the striatum, forming up to several hundred thousand synapses (Matsuda 2009). Striatal DA axons do not form classical closed synapses; DA

is thought to be released from extrasynaptic sites in addition to at synaptic contacts (Pickel *et al.* 1981; Descarries *et al.* 1996), and is proposed to 'spillover' from synaptic release sites (Garris *et al.* 1994; Gonon 1997; Cragg and Rice 2004; Arbuthnott and Wickens 2007). This is because the predominant mechanism for terminating striatal DA signalling is reuptake and recycling of DA via the DAT. Unlike transporters for classical transmitters, DAT is only expressed on DA neurons, not in postsynaptic or glial cells, and has been proposed to be located adjacent to, but not actually within, the presynaptic release site (Freed *et al.* 1995; Nirenberg *et al.* 1996). Additionally, the DAT has a relatively slow turnover rate, much slower than that reported for other transmitter transporters (Wadiche *et al.* 1995; Povlock and Schenk 1997; Cragg and Rice 2004). For these reasons, the DAT is thought to be unable to gate DA within a synapse and DA diffuses away from the release site (Garris *et al.* 1994; Gonon 1997; Cragg and Rice 2004; Arbuthnott and Wickens 2007). Striatal DA signalling is therefore proposed to occur via volume transmission rather than point-to-point transmission.

1.1.3.2 DA function in the striatum

DA functions as a key neuromodulator in the striatum, signalling via metabotropic G-protein coupled receptors (GPCRs). Five DA receptors ($D_{1\text{-}5}$Rs) have been identified, which can be divided into two classes: D_1-like receptors (D_1 and D_5) and D_2-like receptors (D_2, D_3, and D_4), with D_1 and D_2Rs the most abundantly expressed in the striatum (Niznik and Van Tol 1992; Levey *et al.* 1993). D_1-like receptors couple to the G-protein $G_{\alpha s}$, which activates adenylate cyclase (AC), leading to increased cytoplasmic levels of the second messenger cyclic adenosine monophosphate (cAMP). Conversely, D_2-like receptors couple to the G-protein $G_{\alpha i}$, which negatively regulates AC and hence decreases cAMP levels. The βγ subunit of $G_{\alpha i}$ can also have important signalling functions, particularly modulating ion channel activity. D_2Rs exhibit two splice variants, long (D_2L) and short (D_2S) varieties, which couple to slightly different downstream pathways (Guiramand *et al.* 1995). Furthermore, DA receptors can exist in two different affinity states, a high and low affinity state (Wreggett and Seeman 1984; Richfield *et al.* 1989). In the striatum, D_1-like receptors are mostly

found in the low affinity state, whereas D_2-like receptors are mostly found in the high affinity state (Richfield *et al.* 1989).

Different DA receptors are expressed by different cell types within the striatum, which leads to DA signalling having numerous and complex effects on striatal information processing. Firstly, MSNs express high levels of DA receptors, with the two MSN populations expressing different receptor subtypes. dMSNs primarily express D_1Rs, whereas iMSNs primarily express D_2Rs, thought to be largely the D_2L variant (Gerfen *et al.* 1990; Le Moine and Bloch 1995; Usiello *et al.* 2000). There may be a small number of MSNs which co-express both D_1 and D_2Rs (Meador-Woodruff *et al.* 1991; Lester *et al.* 1993; Surmeier *et al.* 1996), but the significance of such cells is controversial; certainly MSNs show largely segregated expression of D_1 and D_2Rs (Bertran-Gonzalez *et al.* 2010). Activation of DA receptors leads to a multitude of changes in the intrinsic and synaptic properties of MSNs via the intracellular signalling pathways described above. D_1R activation increases glutamate receptor surface expression (Yan *et al.* 1999; Dunah and Standaert 2001), decreases voltage-gated Na^+ channel (VGSC) conductance (Surmeier *et al.* 1992), increases L-type voltage-gated Ca^{2+} channel (VGCC) conductance, but decreases conductance via N and P/Q-type VGCCs which couple to Ca^{2+}-activated K^+ channels (Surmeier *et al.* 1995; Vilchis *et al.* 1999). D_2Rs have largely opposing effects, decreasing glutamate receptor currents (Hernández-Echeagaray *et al.* 2004), decreasing L-type VGCC conductance (Hernández-López *et al.* 2000), decreasing VGSC conductance (Surmeier *et al.* 1992) and increasing K^+ conductance (Kitai and Surmeier 1993; Greif *et al.* 1995). The interaction of these modulations, and their overall effect on MSN excitability and synaptic response, is complex and will depend upon the membrane potential of, and inputs to, the MSN. Simplistically however, D_1R activation is proposed to increase the excitability of dMSNs when the membrane potential is depolarised, but decrease excitability when the cells are hyperpolarised, leading to an increased input signal to noise ratio (Surmeier *et al.* 2007). Conversely, the dominant effect of DA signalling in iMSNs is inhibitory, decreasing the excitability of these neurons (Surmeier 2007). In these ways, DA is

thought to play a key role in controlling the balance of activity between the direct and indirect pathways through the basal ganglia.

In addition to these short-term modulatory effects, DA signalling controls the long-term synaptic plasticity of glutamatergic synapses onto MSNs. High frequency electrical stimulation can generate long-term potentiation (LTP) or long-term depression (LTD) of these synapses. LTD is dependent on DA signalling via D_2Rs (Calabresi *et al.* 1997; Kreitzer and Malenka 2005). In iMSNs, D_2R activation drives production of endocannabinoids, which act as a retrograde messenger to decrease presynaptic glutamate release (Kreitzer and Malenka 2005). The mechanism underlying the D_2R-dependence of LTD in dMSNs is still controversial and may be indirect via striatal interneurons (Surmeier 2007). Conversely, LTP, particularly in dMSNs, depends on D_1Rs and their effects on of N-methyl-D-aspartate receptors (NMDARs) (Kreitzer and Malenka 2008). These forms of long-term synaptic plasticity are thought to be key in the reinforcement and procedural learning functions of the striatum (Costa 2007; Lovinger 2010). Hence, striatal DA is thought to be important for such learning.

DA receptors are expressed by many other cell types in the striatum in addition to MSNs. D_2Rs are found on striatal glutamatergic afferents (Wang and Pickel 2002), where they act to reduce glutamate release (Bamford *et al.* 2004). DA receptors are also expressed by at least some classes of GABAergic interneuron (Le Moine *et al.* 1991; Kawaguchi *et al.* 1995), and DA is thought to excite both parvalbumin- and somatostatin-positive interneurons via D_5Rs (Bracci *et al.* 2002; Centonze *et al.* 2003). Additionally, both D_1-like and D_2-like receptors are expressed by ChIs (Le Moine *et al.* 1990; Le Moine *et al.* 1991; Centonze *et al.* 2003). Activation of D_1-like receptors depolarises ChIs through inhibition of a K^+ conductance and activation of a non-selective cation conductance (Aosaki *et al.* 1998; Centonze *et al.* 2003). In contrast, D_2Rs decrease ChI firing and reduce ACh release (DeBoer and Abercrombie 1996; Yan *et al.* 1997; Pisani *et al.* 2000) by modulating VGCC currents (Yan *et al.* 1997), VGSC currents (Maurice *et al.*

2004), the hyperpolarisation-activated Na^+ current (I_h) (Deng *et al.* 2007), and synaptic inputs to these cells (Pisani *et al.* 2000). Therefore, DA can control information processing in the striatum by acting directly on MSNs, but also by acting presynaptically on striatal afferents and by modulating local striatal circuitry.

Finally, D_2Rs, of the D_2S variety (Usiello *et al.* 2000), have a well-characterised negative feedback role on DA neurons themselves. At the level of the DA neuron cell body in the midbrain, D_2 autoreceptors hyperpolarise DA neurons and decrease their firing rate (Bunney *et al.* 1973; Mercuri *et al.* 1997). In the striatum, activation of D_2 autoreceptors on DA terminals decreases DA release and synthesis (Kapatos and Zigmond 1979; Cubeddu and Hoffmann 1982; Mayer *et al.* 1988; Palij *et al.* 1990; Wolf and Roth 1990; Kennedy *et al.* 1992), via modulation of K^+ conductances (Cardozo and Bean 1995; Congar *et al.* 2002; Martel *et al.* 2011), and phosphorylation of TH (Lindgren *et al.* 2001) respectively.

Therefore, DA plays an extensive and varied neuromodulatory role in the striatum. Through the expression of different receptor subtypes and splice variants, in different affinity states, as well as different expression of downstream effector proteins, DA has complex effects on the short- and long-term intrinsic and synaptic properties of striatal neuron populations. DA therefore modulates striatal output directly and via modulation of local striatal circuitry. Simplistically, an increase in extracellular striatal DA is hypothesised to preferentially increase information flow through the direct pathway of the basal ganglia, and hence increase action selection and locomotion, and promote plasticity in striatal MSNs, contributing to reinforcement and procedural learning (Albin *et al.* 1989; Surmeier 2007; Kreitzer and Malenka 2008).

1.1.3.3 DA neuron firing patterns

In vitro, DA neurons exhibit low frequency rhythmic pacemaker activity (Sanghera *et al.* 1984; Grace and Onn 1989). This pacemaker activity is thought to depend upon low threshold VGSCs and VGCCs, in combination with Ca^{2+}- and voltage-gated K^+ conductances (Kang and Kitai 1993;

Wolfart *et al.* 2001; Chan *et al.* 2007; Khaliq and Bean 2010), with the precise conductances involved differing between SNc and VTA DA neurons (Liss *et al.* 2001; Wolfart *et al.* 2001; Chan *et al.* 2007). *In vivo*, this very regular firing is less commonly observed due to the effects of synaptic inputs onto DA neurons. Instead, DA neurons display two main firing patterns: more irregular, low frequency firing (2-8 Hz), and short bursts of high frequency firing (2-5 pulses, usually around 10-40 Hz although firing up to 100 Hz has been reported) (Grace and Bunney 1984; Hyland *et al.* 2002). Burst activity is thought to be driven by the activation of NMDARs on DA neurons by glutamatergic afferents (Charlety *et al.* 1991; Overton and Clark 1992; Christoffersen and Meltzer 1995). Burst frequency and duration is controlled by intrinsic conductances, including Ca^{2+}-activated K^+ channels (Seutin *et al.* 1993; Johnson and Seutin 1997), VGCCs (Shepard and Stump 1999) and the Na^+/K^+ATPase (Johnson *et al.* 1992), as well as by other synaptic inputs including GABAergic connections (Paladini *et al.* 1999).

The origins of the inputs which drive and modulate DA neuron firing are still not well defined. Midbrain DA neurons receive inputs from many diverse brain regions, from the cortex to the brainstem, which vary with DA neuron population (Watabe-Uchida *et al.* 2012). These include excitatory inputs from the cortex, the hypothalamus, the superior colliculus, the lateral tegmental nucleus and the pedunculopontine tegmental nucleus. DA neurons also receive a large inhibitory input from the striatum and other basal ganglia nuclei, the rostromedial tegmental nucleus and the lateral habenula, as well as modulatory inputs from the raphe nucleus and locus coeruleus (Somogyi *et al.* 1981; Hervé *et al.* 1987; Comoli *et al.* 2003; Geisler *et al.* 2007; Watabe-Uchida *et al.* 2012).

1.1.3.4 Behavioural relevance of DA neuron firing patterns

The cortico-basal ganglia loops, and particularly the striatum, are key for action selection, and reinforcement and procedural learning (Costa 2007; Ito and Doya 2011). Striatal DA is important in these processes, since such behaviour is disrupted by dopaminergic lesions (Knowlton *et al.*

1996; Matsumoto *et al.* 1999). Additionally, phasic stimulation of DA neurons, either electrically or more recently selectively using an optogenetic approach, is sufficient to drive behavioural conditioning (Olds and Milner 1954; Tsai *et al.* 2009; Adamantidis *et al.* 2011). Therefore, a role DA neuron burst firing and striatal DA signalling is strongly implicated in reinforcement learning and the behavioural response to reward.

In theoretical models, a reward prediction error is a key parameter underlying reinforcement learning. A reward prediction error is the difference between the reward that is predicted or expected, based on previous experience, and the actual reward delivered or experienced. This value is then used to 'update' reward expectation. One dominant theory, developed largely by Schultz and colleagues, is that DA neuron firing patterns encode such a reward prediction error (**Fig. 1.3**)(Schultz 2007). DA neurons show robust increases in burst firing in response to unexpected rewards, such as food, and the magnitude of the response depends upon the size of the reward (**Fig. 1.3 a**) (Ljungberg *et al.* 1992; Mirenowicz and Schultz 1996; Tobler *et al.* 2005). If animals then learn to associate a neutral cue, such as a light or auditory stimulus, with a subsequent reward, the DA neuron response to the reward decreases with training, as the reward becomes fully predicted by the stimulus. Instead, DA neurons exhibit burst firing in response to the cue, which functions as an unexpected reward (**Fig. 1.3 b**). Conversely, if no reward follows a predictive cue, DA neurons show a decrease in firing rate, indicative of a negative reward prediction error, since the outcome was worse than expected (**Fig. 1.3 c**)(Schultz *et al.* 1993; Schultz *et al.* 1997; Hollerman and Schultz 1998; Waelti *et al.* 2001; Kawagoe *et al.* 2004; Morris *et al.* 2004; Nakahara *et al.* 2004).

However, this theory is still controversial for a number of reasons. The reward prediction error hypothesis suggests that DA neurons should show a decrease in firing in response to aversive stimuli, such as a foot shock or air puff. Indeed, such a response has been reported in the majority of DA neurons (Mirenowicz and Schultz 1996; Ungless *et al.* 2004). However, some

studies have reported that a minority of DA neurons are actually activated by aversive stimuli, or cues predictive of aversive stimuli (Mirenowicz and Schultz 1996; Coizet *et al.* 2006; Brischoux *et al.* 2009; Matsumoto and Hikosaka 2009). An alternative hypothesis therefore suggests that DA neuron bursts simply signal behavioural salience, as opposed to reward, and hence function to shift attention and learn action-outcome associations (Redgrave *et al.* 2008). It is likely that different DA neuron populations have distinct functional roles, which may depend on the location of DA neurons in the midbrain (Brischoux *et al.* 2009; Matsumoto and Hikosaka 2009). Nevertheless, it is universally agreed that switches in DA neuron firing from low frequency firing to high frequency bursts of activity, have strong behavioural relevance.

It is currently not well understood which of the many DA neuron inputs drive behaviourally-significant changes in DA neuron firing. However, recent work has focussed on a role for the pedunculopontine nucleus in driving burst firing (Floresco *et al.* 2003), the superior colliculus in driving visual stimulus-induced changes in firing (Comoli *et al.* 2003; Dommett *et al.* 2005) and the lateral habenula as a source of negative reward signals (Matsumoto and Hikosaka 2007).

It is proposed that reward-related increases in DA neuron burst firing will lead to an increase in striatal DA release; indeed some stimuli which increase DA neuron burst firing, such as novel environments, natural rewards and reward-associated cues, have been shown to increase extracellular DA levels *in vivo* (Roitman *et al.* 2004; Day *et al.* 2007; Stuber *et al.* 2008). As discussed above, such increases in striatal DA will have a multitude of effects on striatal information processing, but are expected to promote action selection and locomotion, and promote plasticity in striatal MSNs, contributing to reinforcement and procedural learning (Albin *et al.* 1989; Surmeier 2007; Kreitzer and Malenka 2008).

However, like all presynaptic terminals, striatal DA terminals dynamically filter action potential patterns into transmitter release. Therefore, striatal DA release, and hence downstream effects on striatal information processing and behaviour, will depend not only on DA neuron firing

patterns but also on factors which modulate the plasticity of DA release. This thesis particularly aims to investigate the presynaptic control of DA release and the short-term plasticity of release, to explore how behaviourally relevant DA neuron firing patterns are dynamically translated into DA release.

1.1.4 Striatal ChIs

Another important striatal transmitter, and one that plays a significant role in controlling DA release, is ACh, released from ChIs. ChIs are large (20-50 µm diameter soma), aspiny neurons, which compose less than 3% of striatal neurons (Kemp and Powell 1971; Graveland and DiFiglia 1985). However, they have relatively extended dendritic trees and dense axonal arborisations (Kawaguchi 1992; Kawaguchi 1993), with huge numbers of vesicle-containing varicosities, presumed to be release sites (Contant *et al.* 1996; Descarries and Mechawar 2000). Indeed, the striatum contains the highest density of cholinergic markers, such as choline acetyltransferase (ChAT) and acetylcholine esterase (AChE) of any brain region (Hoover *et al.* 1978). Therefore, despite their low abundance, each ChI is thought to influence large regions of the striatum and ChIs are hypothesised to play important roles in striatal processing (Calabresi *et al.* 2000). Similarly to striatal DA innervation, ChI varicosities or release sites occur at non-synaptic locations, in addition to at classical synaptic contacts (Contant *et al.* 1996), and many ACh receptors are located outside cholinergic postsynaptic sites (Hersch *et al.* 1994). Therefore, striatal ACh is also thought to act via volume transmission rather than point-to-point transmission (Descarries *et al.* 1997; Descarries and Mechawar 2000).

1.1.4.1 ACh function in the striatum

Similarly to DA, striatal ACh is a neuromodulator with numerous postsynaptic effects. There are two main types of ACh receptor, both of which are found in the striatum: nicotinic receptors (nAChRs) and muscarinic receptors (mAChRs). nAChRs are ligand-gated cation channels, composed of five subunits around a central pore, with the precise ion permeability depending on

the subunit composition. In contrast, mAChRs are GPCRs. There are five classes of mAChRs, $M_{1\text{-}5}$, which can be divided into two families: M_1-like receptors (M_1, M_3 and M_5), which couple to the G-protein $G_{\alpha q}$, resulting in the activation of phosphokinase C and hence increases in the second messengers inositol triphosphate and hence Ca^{2+}, and diacylglyerol, and M_2-like receptors (M_2 and M_4), which couple to $G_{\alpha i}$, resulting in inhibition of AC. M_1, M_2 and M_4Rs are the most abundantly expressed in the striatum (Weiner *et al.* 1990). As with DA signalling, different striatal cell types express different combinations of these receptors, so ACh signalling has diverse downstream effects.

Firstly, mAChRs are expressed postsynpatically on MSNs. M_1Rs are thought to be expressed by the majority of MSNs, both dMSNs and iMSNs (Weiner *et al.* 1990; Yan *et al.* 2001). In general, M_1R activation increases MSN excitability by inhibiting K^+ conductances (Shen *et al.* 2005; Shen *et al.* 2007), inhibiting Ca^{2+} conductances which drive afterhyperpolarisations (Howe and Surmeier 1995; Perez-Rosello *et al.* 2005; Oldenburg and Ding 2011), and enhancing NMDAR-mediated synaptic currents (Calabresi *et al.* 1998). M_1R activation also inhibits endocannabionoid production, which functions as a retrograde messenger to inhibit glutamate release, so M_1Rs also indirectly promote MSN activation by disinhbition of presynaptic glutamate release (Wang *et al.* 2006). M_4Rs are expressed in some iMSNs, but are much more abundantly expressed in dMSNs (Weiner *et al.* 1990; Yan *et al.* 2001). Postsynaptic M_4Rs are thought to be largely inhibitory (Oldenburg and Ding 2011), and are able to modulate the postsynaptic effects of DA signalling (Jeon *et al.* 2010). Again similarly to DA signalling, mAChRs may also modulate the long-term plasticity of glutamatergic synapses onto MSNs. M_1Rs have been suggested to play a role in the initiation of LTD, possibly via interactions with DA signalling or their modulation of endocannabinoid signalling (Calabresi *et al.* 2007). M_1R activation is also required for the induction of striatal LTP, as antagonists completely block this form of plasticity (Calabresi *et al.* 1999).

Secondly, M_2Rs are abundantly expressed on the presynaptic terminals of glutamatergic afferents where they inhibit presynaptic VGCCs and hence reduce transmitter release (Barral *et al.* 1999; Pakhotin and Bracci 2007). ACh receptors are also found on at least some classes of GABAergic interneuron. mAChRs have been reported to inhibit striatal GABA release (Sugita *et al.* 1991; Koós and Tepper 2002), and nAChRs act to depolarize and excite fast spiking GABAergic interneurons (Koós and Tepper 2002). Additionally, ChIs themselves express M_2-like receptors, which function as autoreceptors to decrease ChI firing and ACh release (Schoffelmeer *et al.* 1986; Ding *et al.* 2006), again via modulation of VGCCs (Yan *et al.* 1997; Ding 2006).

Finally, striatal DA terminals express nAChRs (Hill *et al.* 1993; Jones *et al.* 2001). It has been known for some time that in synaptosomal preparations, stimulation of nAChRs can evoke DA release (Grady *et al.* 1992; Marshall *et al.* 1997), and that nAChR antagonists decrease electrically evoked DA release in striatal slices (Zhou *et al.* 2001). It has recently been shown directly that ACh released by synchronous activity in ChIs can act at nAChRs on DA terminals to directly drive DA release, even in the absence of DA neuron activity, both *in vitro* and *in vivo* (Cachope *et al.* 2012; Threlfell *et al.* 2012). In addition however, nAChRs control the short-term plasticity of DA release, determining how DA terminals filter different patterns of activity into DA release. This effect is described and discussed further below. DA neurons also express M_5Rs (Weiner *et al.* 1990). However, it is not currently known whether mAChRs are expressed on DA terminals in the striatum. mAChRs can indirectly control DA release though; muscarinic autoreceptor activation reduces ACh release and hence decreases nAChR activation on DA terminals, indirectly modulating DA release (Threlfell *et al.* 2010).

1.1.4.2 ChI firing patterns and their behavioural relevance

ChIs have relatively depolarised membrane potentials and are characterised by a variety of spontaneous firing patterns *in vivo* (Wilson *et al.* 1990; Aosaki *et al.* 1995) and *in vitro* (Bennett and Wilson 1999), including low frequency tonic, irregular and higher frequency burst firing.

Indeed, many *in vivo* studies simply make recordings from striatal tonically active neurons (TANs), and in the striatum this firing pattern is thought to be characteristic of ChIs such that their identity is usually assumed (Goldberg and Reynolds 2011). This spontaneous activity is governed by intrinsic conductances, including a characteristic I_h, persistent Na^+ conductances, and combinations of VGCCs coupled to Ca^{2+}-activated K^+ conductances (Bennett *et al.* 2000; Goldberg and Wilson 2005).

ChIs, or more correctly TANs, which are assumed to correspond to ChIs, show a relatively high degree of synchronisation of firing (Raz *et al.* 1996). Like DA neurons, TANs show a characteristic change in firing pattern in response to behaviourally salient and reward-related events. This response consists primarily of a pause or suppression of firing lasting 100-300 ms, often flanked by a preceding burst of activity and a rebound burst following the pause (Aosaki *et al.* 1994; Morris *et al.* 2004). This firing pattern has been reported in response to strongly salient unconditioned stimuli, including food rewards and aversive stimuli, but is also found in response to conditioned stimuli predictive of rewarding or aversive events (Aosaki *et al.* 1994; Ravel *et al.* 1999; Morris *et al.* 2004; Apicella *et al.* 2009). This TAN response is therefore coincindent with changes in DA neuron firing in response to reward-related stimuli (Morris *et al.* 2004). However, the behavioural relevance of changes in firing pattern in these two neuronal populations is not identical. DA neuron responses are strongly modulated by reward predictability, or the probability of reward, and hence are thought to encode reward prediction error (Schultz *et al.* 1997; Schultz 2007). While a subset of TANs may encode some aspects of reward prediction error (Ravel *et al.* 2003; Apicella *et al.* 2009), the response of most TANs is not strongly modulated by the probability of reward (Morris *et al.* 2004; Apicella *et al.* 2009). Additionally, TANs tend to show a greater and more consistent response to aversive stimuli than DA neurons (Lee *et al.* 2006; Joshua *et al.* 2008). Also, TANs tend to show a greater synchrony of response than DA neurons (Morris *et al.* 2004). Therefore, whilst the behavioural relevance of TANs, presumed to be ChIs, and DA neuron firing patterns is related, they do not carry redundant information.

A number of possible mechanisms have been proposed to underlie the burst-pause response of ChIs. ChIs receive excitatory inputs from both the parafascicular nucleus of the thalamus and from the cortex (Lapper and Bolam 1992; Thomas *et al.* 2000; Matsumoto *et al.* 2001; Oswald *et al.* 2009), although thalamic inputs are thought to be more numerous (Lapper and Bolam 1992). These inputs are thought to be significant in initiating the burst-pause response (Matsumoto *et al.* 2001; Oswald *et al.* 2009; Ding *et al.* 2010). Stimulation of excitatory inputs, or direct depolarisation of ChIs, causes an initial burst of activity, but leads to deactivation of I_h and persistent Na^+ conductances, and generates a prolonged afterhyperpolarisation mediated by Ca^{2+}-activated K^+ conductances (Reynolds *et al.* 2004; Wilson and Goldberg 2006; Oswald *et al.* 2009). These intrinsic conductances may contribute to the pause response, but additional extrinsic inputs also likely play a role. Stimulation of excitatory striatal afferents results in inhibitory postsynaptic potentials (IPSPs) in ChIs (Suzuki *et al.* 2001), most likely by activating local inhibitory circuitry involving either MSNs or GABAergic interneurons. Additionally, activation of ChIs themselves may augment such GABAergic inhibition, as activation of ChIs is proposed to result in recurrent inhibition of themselves and other ChIs via nAChR-dependent activation of GABAergic interneurons (Sullivan *et al.* 2008). This GABAergic inhibition of ChIs in response to striatal inputs may contribute to the pause in ChI firing. Finally, the pause response is dependent on striatal DA; it is abolished by DA depletion, or D_2 antagonists (Aosaki 1994, Ding 2010). As described in 1.1.3.2, D_2Rs inhibit ChI firing via modulation of Ca^{2+} and Na^+ currents (Yan *et al.* 1997; Maurice *et al.* 2004; Deng *et al.* 2007). Therefore, another possible model for pause generation in ChIs is that thalamic excitatory inputs to ChIs cause ACh release, which acts at nAChRs to drive DA release, which in turn activates D_2Rs to inhibit ChI firing (Ding *et al.* 2010).

The mechanism underlying the rebound excitation response in ChIs following the pause is less well-understood. It has been suggested to be driven by the rebound activation of the I_h current (Aosaki *et al.* 2010). However, this rebound increase in firing is not seen following stimulation of striatal inputs *in vitro* (Ding *et al.* 2010), suggesting that intrastriatal mechanisms are insufficient

to account for the rebound excitation, and that subsequent afferent input may generate this response.

1.1.5 Striatal ACh and DA interactions

Given the coincidence of behaviourally relevant changes in DA neuron and ChI firing, interactions between striatal cholinergic and dopaminergic systems are predicted to be important for the behavioural output of the striatum, including for locomotion and reward-related behaviour and procedural and reinforcement learning. It has long been thought that striatal ACh and DA signalling are essentially antagonistic, and that the output of the striatum reflects a balance between these two neuromodulators (Aosaki *et al.* 2010). This hypothesis originated from the fact that anticholinergic drugs could successfully manage the symptoms of PD (Barbeau 1962), in which striatal DA is lost, as discussed further below. Additionally, DA neurons and ChIs respond to behavioural events with opposing changes in firing rate (Morris *et al.* 2004), and DA and ACh can oppositely affect MSN excitability and synaptic plasticity (Calabresi *et al.* 2000). However, it has become clear in recent years that this hypothesis is oversimplified and that the interactions between striatal DA and ACh are complex, and can be cooperative as well as antagonistic. This can be seen postsynaptically, for example, in dMSNS, both M_1Rs and D_1Rs act to increase excitability and synaptic response (Calabresi *et al.* 2000; Surmeier 2007). Additionally, both ACh and DA inhibit presynaptic glutamate release from striatal afferents (Bamford *et al.* 2004; Pakhotin and Bracci 2007). Finally, as described above, DA and ACh play complex reciprocal roles in modulating the other's release. DA can either increase or decrease ChI activity and ACh release, acting via D_1-like receptors or D_2Rs respectively (Consolo *et al.* 1992; DeBoer and Abercrombie 1996; Yan *et al.* 1997; Pisani *et al.* 2000), and ACh plays a complex role in both driving and modulating DA release (Rice and Cragg 2004; Threlfell *et al.* 2012). Therefore, the interactions between the striatal cholinergic and dopaminergic systems are not simply antagonistic but fairly complex. In this thesis, I have particularly investigated how nAChRs

interact with other presynaptic factors to dynamically control striatal DA release and the short-term plasticity of release.

1.2 Striatal DA release

This thesis aims to explore the presynaptic control of striatal DA release. Our understanding of the mechanisms involved in neurotransmitter release comes largely from studies of peripheral synapses or central glutamatergic synapses, whereas much less is known about the release of modulatory transmitters like DA. DA transmission differs slightly from glutamate transmission. As described above, DA is thought to signal via volume transmission rather than point-to-point synaptic contact (Arbuthnott and Wickens 2007), and DA is proposed to be released from multiple axonal release sites and small varicosities, in addition to at specialised larger synaptic sites (Pickel *et al.* 1996). However, the cellular mechanisms underlying DA release are thought to be similar to those at classical central synapses. Similarly to glutamatergic transmission, DA is stored in small synaptic vesicles in striatal DA terminals (Pickel *et al.* 1996; Pothos *et al.* 1998) and shows Ca^{2+}-dependent, quantal release (Pothos *et al.* 1998).

1.2.1 Mechanisms of transmitter release

Vesicle exocytosis and transmitter release are mediated by essentially the same mechanisms that underlie all cellular membrane fusion. Phospholipid membranes are thermodynamically stable structures. Therefore, vesicle fusion, which disrupts the membrane structure, is an energetically unfavourable process. This energy barrier is overcome by the interactions of three membrane proteins, called SNARE proteins. Two SNARE proteins are located in the plasma membrane, syntaxin-1 and SNAP-25, and one in the vesicle membrane, synaptobrevin. The SNARE proteins contain cytosolic α-helical domains, containing a 60-70 amino acid motif, called the SNARE motif (syntaxin-1 and synaptobrevin each contain one SNARE motif, whereas SNAP-25 has two). These SNARE motifs zip up to form an incredibly stable four helix bundle, the SNARE complex, and draw

the two membranes together. The energy released from this SNARE interaction is thought to be sufficient to overcome the high energy barrier and drive membrane fusion (Südhof and Rothman 2009; Südhof and Rizo 2011). SNARE complexes are then dissociated and the SNARE proteins made available for subsequent reactions, via an ATP-dependent pathway. While SNARE proteins are sufficient to drive membrane fusion *in vitro*, many other proteins are necessary for membrane fusion *in vivo*, for example Sec-1/Munc-18-like proteins, which may promote SNARE complex formation or control membrane curvature (Südhof and Rizo 2011).

At synapses, vesicle fusion is tightly regulated and strongly Ca^{2+}-dependent (Katz and Miledi 1967). Membrane depolarisation opens presynaptic VGCCs. This allows Ca^{2+} influx down its electrochemical gradient, creating local presynaptic microdomains of high Ca^{2+} concentration. Ca^{2+} binds cooperatively to the exocytotic Ca^{2+} sensor, thought to be the vesicle protein synaptotagmin (Chapman 2002), which drives full SNARE complex formation and vesicle fusion, releasing the stored neurotransmitter into the extracellular space (Sudhof 2004).

This Ca^{2+}-dependent vesicle fusion is incredibly rapid, and tightly regulated, due to the actions of a huge array of presynaptic proteins. Large, multidomain, active zone proteins such as RIMs, Munc-13 and α-liprin, interact with vesicle proteins, the cytoskeleton and VGCCs (Thomson 2000; Sigrist and Schmitz 2011; Südhof and Rizo 2011). These interactions hold synaptic vesicles at the presynaptic active zone, ready for rapid release. They also hold VGCCs in close proximity to docked synaptic vesicles, allowing rapid local increases in Ca^{2+}. Such interactions are thought to result in very rapid synchronous transmitter release, within around 200 µs of membrane depolarisation (Sabatini and Regehr 1996). Such active zone proteins are also thought to 'prime' synaptic vesicles for release, possibly by 'unlocking' SNARE proteins from other protein-protein interactions (Augustin *et al.* 1999; Koushika *et al.* 2001). Many smaller presynaptic proteins also regulate vesicle exocytosis. For example, Rab3 proteins are thought to regulate vesicle docking and fusion (Geppert *et al.* 1997) and complexin proteins are thought to have a dual function,

promoting SNARE complex formation, but clamping it before full membrane fusion (Tang *et al.* 2006; Brose 2008).

In summary, rapid, synchronous, Ca^{2+}-dependent neurotransmitter release occurs in response to membrane depolarisation, and this process is regulated by a large array of presynaptic proteins and their interactions.

1.2.1.1 Vesicle pools

However, at the majority of central synapses studied to date, only a small minority of synaptic vesicles are docked and primed for release as described above. Instead, at many synapses, vesicles are thought to be organised into different vesicle pools (Rizzoli and Betz 2005). This is based on anatomical evidence, showing that vesicles are found at varying distances from the presynaptic membrane (Schikorski and Stevens 1997), and functional evidence, that transmitter release in response to different stimulation protocols or prolonged stimulation shows multiple kinetic phases, suggesting that not all vesicles are equally releasable (Elmqvist and Quastel 1965; Pieribone *et al.* 1995; Stevens and Tsujimoto 1995). Central glutamatergic synapses are proposed to have three main vesicle pools: a readily releasable pool, which consists of docked vesicles immediately available for release, a recycling pool, from which vesicles are released following longer trains of physiological stimulation, and a reserve pool, which can be mobilised to supply the recycling pool following prolonged stimulation. Only 1-2% of synaptic vesicles are thought to be readily releasable, with 15-20% in the recycling pool and around 80% in the reserve pool (Rizzoli and Betz 2005). Much less is understood about the organisation of synaptic vesicles in striatal DA terminals. Vesicles in DA terminals do not show clear clustering into distinct pools but are distributed throughout axon varicosities (Nirenberg *et al.* 1997; Anwar *et al.* 2011). However, functional vesicle pools do not always reflect spatial organisation (Rizzoli and Betz 2005), and it has been proposed that DA terminals do contain at least two vesicle pools, a recycling pool and a reserve pool. This is because the decay in electrically stimulated DA release following inhibition

of DA synthesis occurs with two distinct kinetic phases (Javoy and Glowinski 1971), suggesting that DA vesicles exist in two different states. Additionally, repeated stimulation of DA release following DA synthesis inhibition eventually abolishes DA release, but only decreases striatal DA content by around 30-40%, suggesting the presence of a non-releasable store of DA (Ewing *et al.* 1983). Finally, certain pharmacological agents such as amfonelic acid and cocaine can promote DA release, even when DA release has been depleted following inhibition of DA synthesis, again suggesting the presence of a second store of DA which can be mobilised by these drugs (Ewing *et al.* 1983; Venton *et al.* 2006). However, a recent study using fluorescently labelled exogenous transmitter to monitor DA release in brain slices, found that destaining kinetics showed only a single exponential phase, suggesting that there was only one functional vesicle pool (Gubernator *et al.* 2009). Overall it appears likely that striatal DA terminals do contain multiple functional vesicle pools, but their function and organisation is incompletely understood.

1.2.2 Short-term plasticity of transmitter release

In this thesis I am particularly interested in exploring the dynamic nature of striatal DA release, and hence how different firing patterns are translated into different levels of DA release. Transmitter release at all synapses is highly dynamic or plastic; each action potential that arrives at a presynaptic terminal does not release the same number of synaptic vesicles. Plasticity of transmitter release can occur over multiple timescales, from milliseconds to minutes (Thomson 2000; Zucker and Regehr 2002). Here I am particularly interested in exploring the short-term plasticity of release, over a timescale of milliseconds, since this will affect DA release evoked by burst vs. low frequency activity. Over this timescale, transmitter release can be facilitated, where an action potential evokes more transmitter release than the preceding one, or depressed, such that an action potential evokes less release than the preceding one (Thomson 2000; Zucker and Regehr 2002). These processes, and the mechanisms underlying them, have again been primarily explored at certain central glutamatergic synapses.

1.2.2.1 Facilitation

Many synapses show short-term facilitation of release, which decays with a time constant of around 10-100 ms (Thomson 1997; Markram *et al.* 1998). This is thought to be largely due to residual Ca^{2+} within the presynaptic terminal (Katz and Miledi 1968; Charlton *et al.* 1982; Regehr *et al.* 1994). A first action potential invades the terminal, driving Ca^{2+} entry VGCCs, and evoking vesicle fusion as described above. When a second action potential arrives in quick succession, some Ca^{2+} from the first action potential remains in the presynaptic cytosol, or bound to the Ca^{2+} sensor. This increases the total presynaptic Ca^{2+} concentration, and hence increases transmitter release. There is much evidence that this residual Ca^{2+} mechanism underlies facilitation at the majority of synapses. To begin with, the mechanism of facilitation must be upstream of transmitter release, as facilitation can be seen in the absence of initial release (del Castillo and Katz 1954; Thomson *et al.* 1993), but downstream of Ca^{2+} entry, as at some synapses facilitation can be evoked under voltage-clamp conditions where Ca^{2+} entry is fixed (Charlton *et al.* 1982). Additionally, intraterminal Ca^{2+} chelators reduce facilitation (Regehr *et al.* 1994; Atluri and Regehr 1996), and facilitation correlates with presynaptic Ca^{2+} concentration (Delaney and Tank 1994; Regehr *et al.* 1994). Other mechanisms, however, may contribute to the short-term facilitation of release. At some synapses, activity-dependent changes in ion conductances are thought to play a role. P/Q-type VGCCs, a class of VGCC often expressed presynaptically, show Ca^{2+}-dependent facilitation (Van Petegem and Minor 2006). Therefore, the Ca^{2+} influx evoked by an initial action potential enhances Ca^{2+} currents evoked by subsequent action potentials, which may drive increased release at some synapses (Mochida *et al.* 2008). At other terminals, activity-dependent changes in conductance may alter action potential shape and hence contribute to the facilitation of release. For example, activity-dependent inactivation of K^+ channels can slow repolarisation, broadening the action potential and hence prolonging the period of Ca^{2+} entry and increasing Ca^{2+}-dependent release (Geiger and Jonas 2000). Therefore multiple mechanisms, with multiple timescales, can combine to drive short-term facilitation of transmitter release.

1.2.2.2 Depression

Alternatively, synapses can show short-term depression of release, where subsequent action potentials evoke less transmitter release than a first. This can occur over multiple timescales, from milliseconds to seconds; indeed there are often several components of depression at a given synapse, with different time constants (Thomson 2000; Zucker and Regehr 2002). As with facilitation, there can be multiple mechanisms underlying this decrease in release. Firstly, the traditional model of depression is a release-dependent 'depletion' model (Elmqvist and Quastel 1965). This proposes that transmitter release depletes the terminal of vesicles. Initially, following single or very few action potentials, the terminal may be depleted of docked, immediately releasable vesicles, and then with longer stimulations the readily releasable pool of vesicles may be depleted. A slightly alternative model proposes that release from a given release site makes the release site itself refractory, preventing subsequent release from the same site for a few tens of milliseconds (Thomson 2000). In either case, these depletion or refractory models are strictly release-dependent; there is no depression without release, and depression increases with increased initial release. These proposed mechanisms of depression are supported by statistical models of synaptic function, where transmitter release is modelled by a Poisson distribution with the parameter *n* representing the number of vesicles or release sites available, and the parameter *p* representing release probability. Such models demonstrate that depression is often best accounted for by a decrease in the parameter *n* (Bennett and Florin 1974), supporting a depletion model, although decreases in *p* have also been reported (Betz 1970). Additionally, the majority of synapses explored to date show an inverse relationship between P_r and subsequent release, further supporting this model of depression (Manabe *et al.* 1993; Thomson *et al.* 1993; Debanne *et al.* 1996; Dobrunz and Stevens 1997; Ouardouz and Lacaille 1997; Thomson 1997). Where P_r is high, an initial action potential evokes much transmitter release, depleting the terminal of immediately releasable vesicles or causing large numbers of release sites to become refractory, hence reducing release evoked by a subsequent action potential. Conversely, where

P_r is low, few vesicles are released by an initial action potential, leaving many vesicles available for subsequent release and hence the synapse shows little depression. This inverse relationship can be seen either when P_r is changed at a given synapse, for example by changing the extracellular concentration of Ca^{2+} ($[Ca^{2+}]_o$)(Manabe *et al.* 1993; Thomson *et al.* 1993; Thomson 1997), or across synapses with varying P_r (Debanne *et al.* 1996; Dobrunz and Stevens 1997).

In some situations, however, synapses can express a release-independent form of depression. Here, there is no inverse relationship between P_r and subsequent release, suggesting that depression is independent of initial release (Thomson and Bannister 1999; Thomson 2000). Multiple mechanisms have been proposed to underlie this form of plasticity, and the exact mechanism, or combination of mechanisms is likely synapse specific. As with facilitation, activity-dependent changes in ion conductances may play a role. For example, some VGCCs show Ca^{2+}-dependent inhibition (Van Petegem and Minor 2006), so Ca^{2+} influx in response to subsequent action potentials is reduced, which may contribute to depression at some synapses (Forsythe *et al.* 1998; Xu and Wu 2005). Alternatively, some terminals express Ca^{2+}-activated K^+ channels which might be activated following an initial action potential. This will enhance repolarisation of subsequent action potentials, leading to reduced Ca^{2+} entry and transmitter release (Dodson and Forsythe 2004). Another suggested cause of release-independent depression is axon conductance failure, where the probability of subsequent action potentials invading the presynaptic terminal is reduced, due to changes in membrane conductance (Brody and Yue 2000; Soleng *et al.* 2004).

Therefore, the short-term plasticity of transmitter release exhibited by a synapse is highly complex, and can be composed of multiple independent components operating over multiple timescales. The short-term plasticity exhibited will depend on vesicle numbers and organisation, ion channel expression and density, P_r, and multiple other presynaptic factors, including the

expression of presynaptic proteins involved in controlling vesicle trafficking and fusion described above.

1.2.2.3 Short-term plasticity of DA release

Much less is understood about the short-term plasticity of release of modulatory transmitters, such as DA. In studies modelling DA release *in vivo*, it has often been assumed that each action potential in a DA neuron evokes a constant amount of DA release in the striatum (Wightman *et al.* 1988; Wightman and Zimmerman 1990; Garris *et al.* 1994; John and Jones 2007). However, several results suggest that DA release *in vivo* exhibits multiple forms of short-term plasticity like other transmitter systems. For example, Chergui et al. report facilitation of DA release during burst stimulation, with subsequent action potentials in a burst releasing more DA than the initial stimulation (Chergui *et al.* 1994). A more recent study suggests that DA release *in vivo* is highly dynamic and displays at least three plasticity components, a facilitatory component with a time constant of several seconds, and two depression components, with time constants in the second and minute range respectively (Montague *et al.* 2004). It is difficult to investigate the plasticity of DA release over shorter timescales *in vivo*, as DA release evoked by short stimulation trains *in vivo* tends to be low. In addition, the intact circuitry of the *in vivo* model makes it hard to tease apart network and presynaptic factors influencing the short-term plasticity of DA release.

In vitro, DA release shows strong paired-pulse depression, which recovers over a 0.5-2 minute time course. One component of this is likely due to the activation of D_2 autoreceptors (Kennedy *et al.* 1992; Phillips *et al.* 2002), but strong depression is still exhibited even in the presence of D_2R antagonists (Mayer *et al.* 1988; Kennedy *et al.* 1992; Cragg 2003). The mechanisms underlying this are not well understood, but have been suggested to involve depletion of recycling vesicles (Abeliovich *et al.* 2000).

1.2.3 Control by network factors

In addition to intrinsic presynaptic factors, network factors can also modulate transmitter release and its short-term plasticity via receptors expressed on presynaptic terminals. Firstly, many presynaptic terminals express autoreceptors, such that transmitter release feeds back onto its own presynaptic terminal, usually resulting in a decrease in subsequent release, and a form of presynaptic depression. As described in 1.1.3.2, DA acts at D_2 autoreceptors on DA terminals to inhibit DA synthesis and release (Kapatos and Zigmond 1979; Cubeddu and Hoffmann 1982; Mayer *et al.* 1988; Palij *et al.* 1990; Kennedy *et al.* 1992), and hence these receptors contribute to the short-term depression of DA release over a timescale of 250 ms up to several seconds (Kennedy *et al.* 1992; Benoit-Marand *et al.* 2001; Phillips *et al.* 2002).

Secondly, terminals can express heteroceptors, such that release is controlled by other transmitters. Heteroceptors can be either metabotropic GPCRs, which modulate release over relatively long timescales of hundreds of milliseconds to seconds, or ionotropic receptors, which can affect transmitter release over tens of milliseconds. As described in 1.1.4.1, an important heteroceptor found on DA terminals is nAChRs (Hill *et al.* 1993; Jones *et al.* 2001). ACh from ChIs can act at nAChRs to directly drive DA release (Threlfell *et al.* 2012) (**Fig. 1.4**). However, nAChRs also significantly control the short-term plasticity of DA release. In drug-free conditions, when nAChRs are active, DA release *in vitro* shows strong short-term depression; high frequency burst electrical stimulation does not release much more DA than single electrical stimulus pulses or low frequency trains (Rice and Cragg 2004). Inhibition of nAChRs decreases DA release evoked by single stimulus pulses or low frequency activity (Zhou *et al.* 2001), consistent with the role of nAChRs in driving DA release. However, nAChR inhibition also relieves short-term depression, promoting release by subsequent activity or high frequency bursts (Rice and Cragg 2004; Zhang and Sulzer 2004) (**Fig. 1.5**). Therefore nAChRs on DA terminals have complex effects on DA

release, not simply driving DA release but modulating how DA neuron firing patterns are filtered into release.

It is not currently known whether striatal DA terminals express other heteroceptors. DA neurons do express M_5 mAChRs (Weiner *et al.* 1990) and metabotropic glutamate receptors (mGluRs) (Testa *et al.* 1994) and both mAChRs and mGluRs can modulate DA release (Lehmann and Langer 1982; Hu *et al.* 1999; Zhang *et al.* 2002; Zhang and Sulzer 2003). Additionally, DA release from synaptosomal preparations has been reported to be modulated by ionotropic glutamate receptors (Chéramy *et al.* 1996; Grilli *et al.* 2012). However, the effects of mAChRs and mGluRs on DA release may be indirect (Avshalumov *et al.* 2003; Threlfell *et al.* 2010) and there is little anatomical evidence for ionotropic glutamate receptors on striatal DA terminals (Bernard *et al.* 1997; Bernard and Bolam 1998). Certainly, glutamate and GABA receptors do not modulate DA release evoked by subsecond stimuli *in vitro* (Avshalumov *et al.* 2003; Cragg 2003).

Finally, diffusible messengers can control transmitter release. For example, in the striatum, hydrogen peroxide released from MSNs acts retrogradely to control DA release (Avshalumov *et al.* 2003).

Therefore the short-term plasticity of striatal DA release is highly dynamic and controlled by a complex mix of presynaptic and network factors.

1.3 Pathology

As discussed above, striatal DA plays a key role in many behaviours, from locomotion to reinforcement learning. For this reason, dysfunction in striatal DA signalling can cause many different pathological symptoms. Dysfunctions in striatal DA are thought to underlie a number of neurological and psychological disorders including PD and drug addiction, and may contribute to many more including attention deficit hyperactivity disorder (ADHD) and schizophrenia (Albin *et*

al. 1989; Koob 1992; Olanow and Tatton 1999; Everitt and Wolf 2002; Meisenzahl *et al.* 2007; del Campo *et al.* 2011). In addition to investigating the physiological control of striatal DA release, which may have implications for these disorders, this thesis has specifically explored the effects of cocaine, a significant drug of abuse, and mutations in α-syn, a protein implicated in the pathogenesis of PD, on striatal DA release.

1.3.1 Parkinson's disease

PD is a progressive neurodegenerative disorder, with a prevalence of approximately 1% in over 60s (de Rijk *et al.* 2000). PD is usually considered primarily a motor disorder with cardinal symptoms of bradykinesia, rigidity and resting tremor (Samii *et al.* 2004). However non-motor symptoms are also common and can include cognitive decline, depression and other mood disorders, sleep disorders and autonomic dysfunction (Aarsland *et al.* 2008; O'Sullivan *et al.* 2008; Stacy 2011). The motor symptoms of PD are thought to be primarily due to the degeneration of nigrostriatal DA neurons and the consequent loss of striatal DA. DA neurons in the ventral tier of the SNc, which preferentially innervate dorsolateral sensorimotor-associated areas of the striatum, as described above, are selectively vulnerable to degeneration in PD, whereas DA neurons of the SNc dorsal tier and VTA, which preferentially innervate the ventral striatum, are relatively spared (Fearnley and Lees 1991). According to the classic model of basal ganglia function, this reduction in dorsal striatal DA causes an imbalance in the motor-associated direct and indirect pathways of the basal ganglia, leading to deficits in motor function (Albin *et al.* 1989). More recent data suggests that the lack of striatal DA also results in pathological synchronisation of activity in the basal ganglia, which may also contribute to the deficits in motor function (Brown 2003).

PD is largely an idiopathic disease, thought to be caused by a complex interplay of genetic and environmental factors (Samii *et al.* 2004). However, around 10-20% of PD cases are strongly familial (Alberio *et al.* 2012), and several genetic loci have now been linked to PD. The first of

these to be identified was *SNCA*, the gene encoding the protein α-syn. Mutations in or multiplication of *SNCA* cause dominantly inherited PD (Polymeropoulos *et al.* 1997; Krüger *et al.* 1998; Singleton *et al.* 2003; Zarranz *et al.* 2004). Other genes linked to familial PD include *LRRK2*, encoding a protein kinase of unknown function, *PARK7*, encoding the chaperone DJ-1, *PINK1,* encoding a mitochondrial kinase, and *PARK2*, which encodes a ubiquitin ligase (Corti *et al.* 2011). Most attention has focussed on α-syn, since, unlike several of these proteins, α-syn is also implicated in idiopathic PD. Genome wide association studies have linked polymorphisms in *SNCA* with idiopathic PD (Satake *et al.* 2009; Simon-Sanchez *et al.* 2009) and α-syn is the major component of Lewy bodies, the protein aggregates which are the pathological hallmark of the disease (Spillantini *et al.* 1997). Therefore, α-syn is strongly implicated in the pathogenesis of PD.

The underlying mechanisms of cell death, and the pathways which link α-syn to DA neuron degeneration in PD, are not fully characterised. Multiple interconnected cellular pathways have been implicated, including mitochondrial dysfunction, oxidative stress, proteasomal dysfunction and lysosomal dysfunction (Bandopadhyay and de Belleroche 2010; Imai and Lu 2011; Shulman *et al.* 2011). Much recent interest, however, has focussed on presynaptic dysfunction as a key early event in PD pathogenesis, since decreases in striatal DA levels, and degeneration of striatal DA terminals, precede overt degeneration of DA neurons in the midbrain (Hornykiewicz 1998; Cheng *et al.* 2010). Additionally, the primary function of α-syn is thought to be presynaptic.

There are 3 members of the synuclein family, α, β and γ, which may exhibit some functional redundancy (Senior *et al.* 2008; Anwar *et al.* 2011). α-syn is a small protein expressed presynaptically throughout the central nervous system (Maroteaux *et al.* 1988). It has been implicated in a number of functions, from neuroprotection (Chandra *et al.* 2005) to enzyme regulation (Perez *et al.* 2002), but most recent work has focused on its role in controlling presynaptic vesicle dynamics (Nemani *et al.* 2010). Knockdown or deletion of α-syn causes changes in synaptic vesicle organisation and transmitter release in non-dopaminergic neurons

(Murphy *et al.* 2000; Cabin *et al.* 2002). In the nigrostriatal DA system, synuclein knockout causes increases in striatal DA release (Senior *et al.* 2008; Anwar *et al.* 2011) or changes in the plasticity of DA release (Abeliovich *et al.* 2000; Yavich *et al.* 2004). Conversely, overexpression of wild-type (WT) or mutant α-syn inhibits transmitter release from non-neuronal cells (Park *et al.* 2002; Larsen *et al.* 2006), causes deficits in vesicle cycling and transmitter release in cultured neurons (Nemani *et al.* 2010), and modulates striatal DA release in response to prolonged stimulations *in vivo* (Yavich *et al.* 2005).

Therefore, α-syn may be one presynaptic protein that contributes to the control of striatal DA release and release plasticity, and dysfunction of this protein may be significant in early deficits in striatal DA and the early pathogenesis of PD. However, the effects of mutant α-syn on striatal DA release, prior to DA neuron degeneration, remain incompletely explored. I have investigated this in chapter 6 of this thesis.

1.3.2 Drug addiction

Drug addiction or dependence is a debilitating disorder, characterised by compulsive drug-taking and seeking, at the expense of other activities and despite adverse effects. The striatum, and particularly its dopaminergic innervation, is strongly implicated in the pathogenesis of addiction (Koob 1992; Everitt and Wolf 2002).

Goal-directed behaviour and reinforcement learning, with regards to natural rewards such as food, are thought to strongly depend on the NAc and its dopaminergic input, since lesions of the NAc core (NAcC) or DA antagonist administration prevent reinforcement learning, and inhibit previously acquired goal-directed responses for food and water (Wise *et al.* 1978; Wise and Schwartz 1981). Such learning is proposed to occur via DA-dependent changes in the long-term synaptic plasticity at striatal synapses (Wise 2004). More recent work has also suggested a role for the dorsal striatum and its dopaminergic innervation in learned behavioural responses,

particularly in habit formation and stimulus-response learning (Barnes *et al.* 2005; Yin 2006; Graybiel 2008).

One influential hypothesis of drug addiction suggests that addictive drugs hijack these learning mechanisms to strongly reinforce drug-associated cues and drug-taking (Everitt *et al.* 2001). Animals will self-administer drugs of abuse directly into the NAc (Olds 1982; Carlezon *et al.* 1995) and lesions of the DA input to the NAc, or administration of DA antagonists into the NAc, inhibit drug self-administration (Roberts *et al.* 1977; Phillips *et al.* 1994). Recent work also suggests that DA signalling in the dorsal striatum appears to be necessary for later developing, habitual drug-taking behaviour (Belin and Everitt 2008). Together, such results strongly implicate the striatum and its dopaminergic innervation in the pathogenesis of drug addiction. Most drugs of abuse increase extracellular DA in the NAc, via a variety of mechanisms. It is thought that this increase in DA in the NAc has long-term effects on information processing in MSNs, contributing to the reinforcing and eventually addictive effects of these drugs (Wise 2004). The effects on DA transmission of the two drugs most relevant to this thesis are described below:

1.3.2.1 Nicotine

Nicotine is an nAChR agonist and can increase DA release in the NAc by promoting midbrain DA neuron firing, via actions on several different types of midbrain neuron. Firstly, in the midbrain, nicotine is thought to act at nAChRs on DA neurons themselves to directly increase DA neuron firing rate and/or burst firing activity (Pidoplichko *et al.* 1997; Mameli-Engvall *et al.* 2006). Secondly, nicotine increases excitatory input to VTA DA neurons, acting at nAChRs on glutamatergic terminals to promote glutamate release (Mansvelder and McGehee 2000; Pidoplichko *et al.* 2004). Finally, nAChRs are found on GABAergic afferents to DA neurons. Nicotine levels found in smokers are proposed to be sufficient to desensitise this class of nAChR, leading to disinhibition and increased firing of DA neurons (Mansvelder *et al.* 2002; Pidoplichko *et al.* 2004). In addition to their effects on DA neuron firing, nAChRs are also located on DA

terminals in the striatum, as described above, where nicotine may act to directly modulate striatal DA release. Nicotine may act as an agonist in the striatum to directly drive striatal DA release (Rapier *et al.* 1988; Threlfell *et al.* 2012). However, nicotine levels found in smokers are thought to desensitise nAChRs found on DA terminals (Zhou *et al.* 2001). As described above, this will have complex effects on striatal DA release, decreasing DA release evoked by low frequency activity but increasing that evoked by high frequency bursts (Rice and Cragg 2004; Zhang and Sulzer 2004). Therefore, nicotine is proposed to increase the contrast between DA release evoked by high frequency bursts of DA neuron activity in response to salient or reward-related events, and that evoked by low frequency tonic activity (Cragg 2006). This may increase DA-mediated reinforcement of sensory cues, and contribute to the reinforcing effects of nicotine. However, the effects of nAChRs on striatal DA release are not fully explored. In this thesis, I have explored how nAChRs interact with other presynaptic factors to control striatal DA release and the short-term plasticity of release, which will have implications for the actions of nicotine on striatal DA release.

1.3.2.2 Cocaine

The major target of cocaine and other psychostimulant drugs is the DAT, although cocaine also binds to several other targets including the noradrenaline and serotonin transporters (NET and SERT respectively) (Ritz *et al.* 1990). A key role for the DAT in the reinforcing effects of cocaine is supported by the fact that the reinforcing effects of cocaine analogues correlate with their binding to the DAT (Ritz *et al.* 1987), and that the reinforcing effects of cocaine are absent in mice expressing a cocaine-insensitive DAT (Chen *et al.* 2006). Cocaine inhibits the DAT, preventing DA reuptake from the extracellular space, and hence increasing and prolonging extracellular DA signals (Wieczorek and Kruk 1994). This is proposed to modulate the synaptic strength of inputs both onto DA neurons in the midbrain, promoting increased DA neuron firing (Ungless *et al.* 2001; Saal *et al.* 2003; Borgland *et al.* 2004), and onto MSNs in the NAc (Boudreau

and Wolf 2005; Kourrich *et al.* 2007; Kasanetz *et al.* 2010), contributing to the reinforcing properties of the drug.

However, the DAT plays complex roles in shaping striatal DA signalling, so the effects of psychostimulant drugs such as cocaine on striatal DA signalling are not straightforward. Due to the perisynaptic location and slow turnover rate of the DAT, described above, DA uptake limits DA signals evoked by low frequency activity to a greater extent than that evoked by high frequency bursts (Garris and Rebec 2002). Furthermore, by prolonging extracellular DA signals, DAT inhibition increases activation of D_2Rs on DA terminals and ChIs, in addition to on MSNs. This causes an inhibition of DA synthesis (Galloway 1990), and, as shown by previous work in our lab, modulation of DA release and the short-term plasticity of release (Clements *et al.*). In addition, DAT inhibition has been suggested to increase DA release, possibly by mobilising a reserve pool of DA vesicles (Stamford *et al.* 1989; Lee *et al.* 2001; Robinson and Wightman 2004; Venton *et al.* 2006). Therefore, DAT inhibitors like cocaine disrupt striatal DA signalling in complex ways in addition to simply prolonging extracellular DA signals.

The complex effects of the DAT on striatal DA release and the short-term plasticity of release have not been fully explored. In this thesis I will explore how the DAT can control DA release plasticity, both when nAChRs are active and inhibited. This will have implications for the actions of psychostimulant drugs of abuse like cocaine.

1.4 Summary

Striatal DA is important for many behaviours including goal-directed actions and behavioural conditioning, and dysfunctions in striatal DA signalling contribute to a number of diseases, including PD and drug addiction. It is proposed that increases in DA neuron burst firing, which strongly correlate with salient and reward-related behavioural events, will increase striatal DA levels, modulating information processing in the striatum, and hence behavioural output.

However, striatal DA release is dynamically controlled by many factors acting at the level of DA terminal, in addition to DA neuron firing pattern. Therefore, to fully understand the behavioural relevance of DA neuron firing patterns, and how DA signalling is disrupted in disease, it is necessary to understand the presynaptic control of DA release. In this thesis I have used fast-scan cyclic voltammetry (FCV) in acute murine striatal slices to investigate the presynaptic control of striatal DA release. The methods used throughout this thesis will be described in chapter 2. I will begin in chapter 3 by investigating the interaction between P_r and nAChRs in dynamically controlling DA release. Chapter 4 will explore a previously unidentified role for the DAT in controlling DA release plasticity, when nAChRs are inhibited. Chapter 5 will explore regional differences in the previously identified role of the DAT in controlling DA release plasticity, via control of D_2R activation, when nAChRs are active. Finally, in chapter 6, I will investigate how overexpression of a mutant presynaptic protein, α-syn, modulates striatal DA release in a murine model of PD

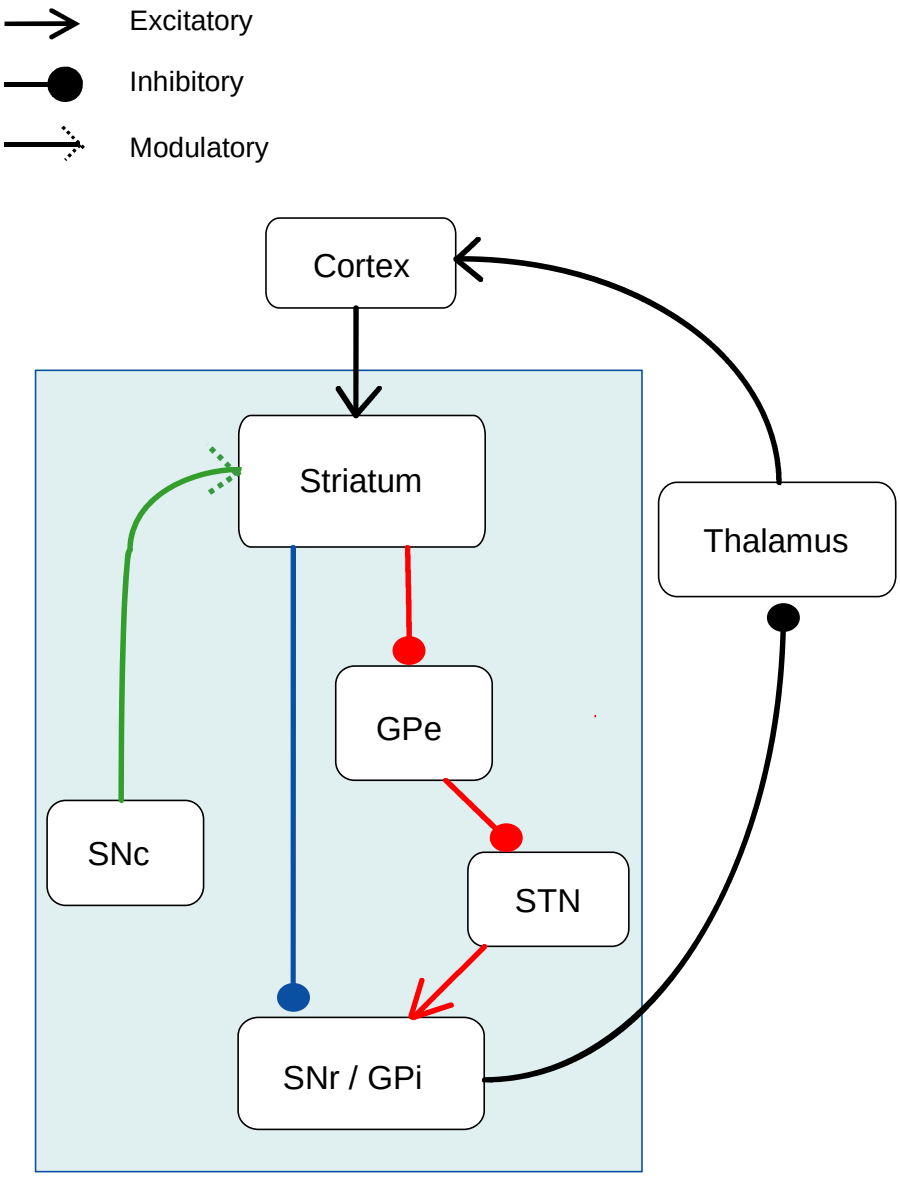


Figure 1.1 Classic direct and indirect pathways of the basal ganglia. Blue rectangle: basal ganglia. Blue connections: direct pathway, Red connections: indirect pathway Green connections: DA innervation

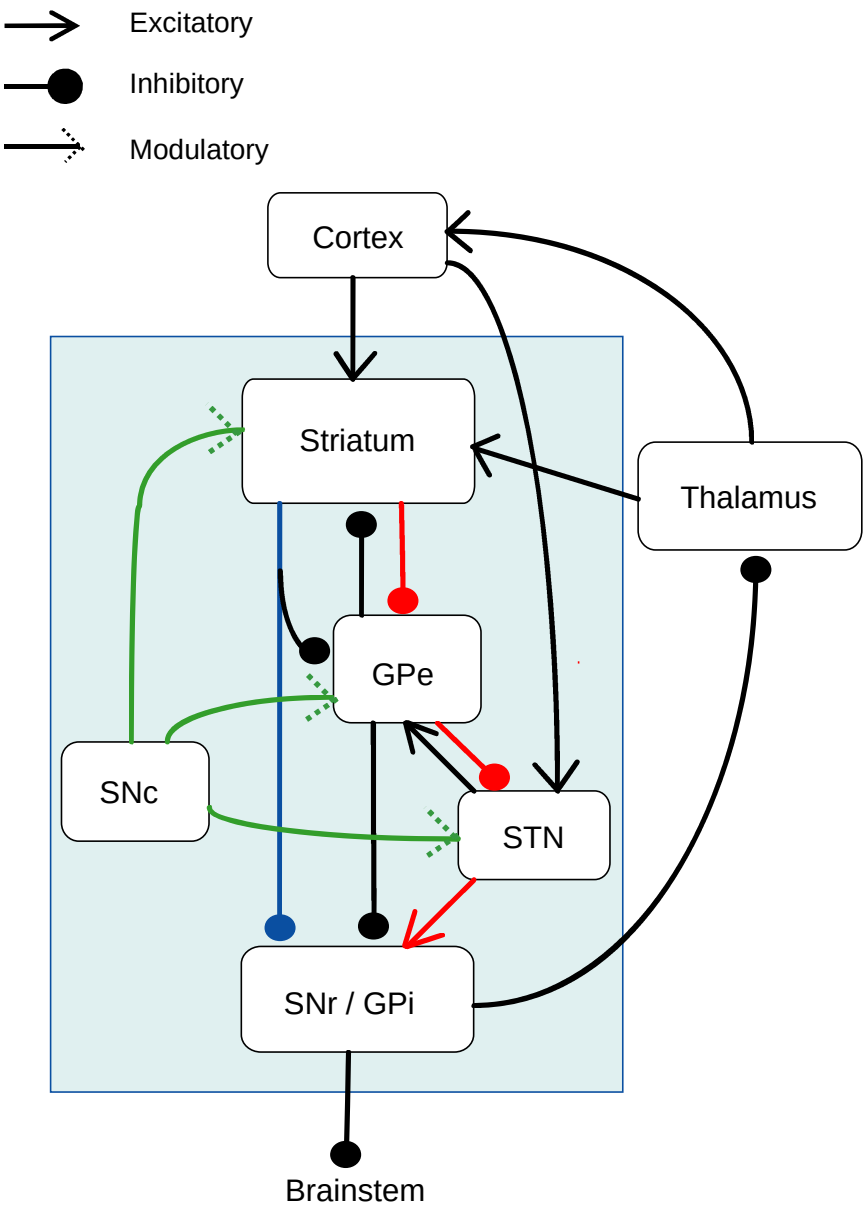


Figure 1.2 More complete model of the basal ganglia. Blue rectangle: basal ganglia. Blue connections: direct pathway, Red connections: indirect pathway Green connections: DA innervation

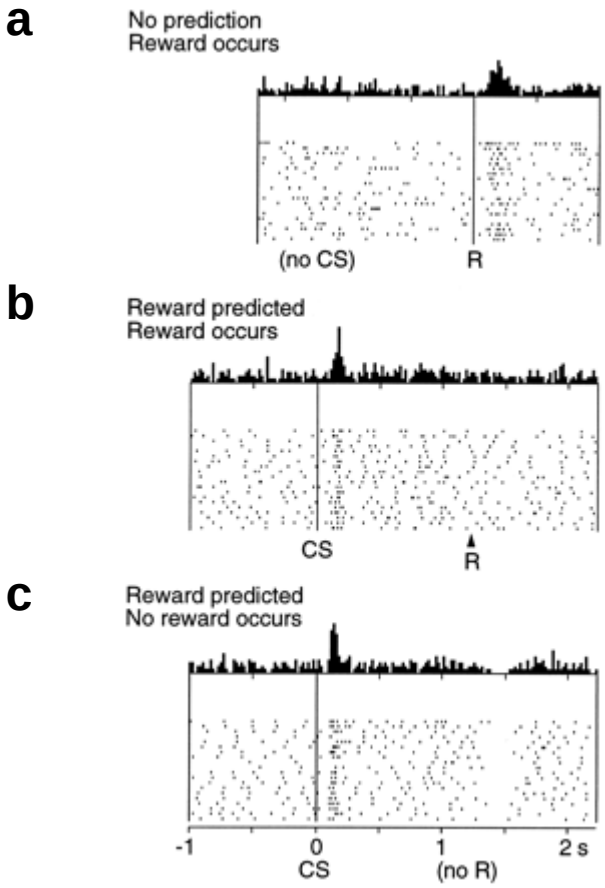


Figure 1.3 Dopamine neurons are proposed to encode a reward prediction error. R = reward delivery, CS = conditioned stimulus delivery (i.e. neutral cue such as light or auditory stimulus, which has been previously associated with reward delivery) **(a)** Unexpected reward drives increase in DA neuron firing. **(b)** Conditioned stimulus drives increase in DA neuron firing but fully predicted reward does not. **(c)** Reward omission following conditioned stimulus decrease DA neuron firing. Figure from Schultz et al. 1997.

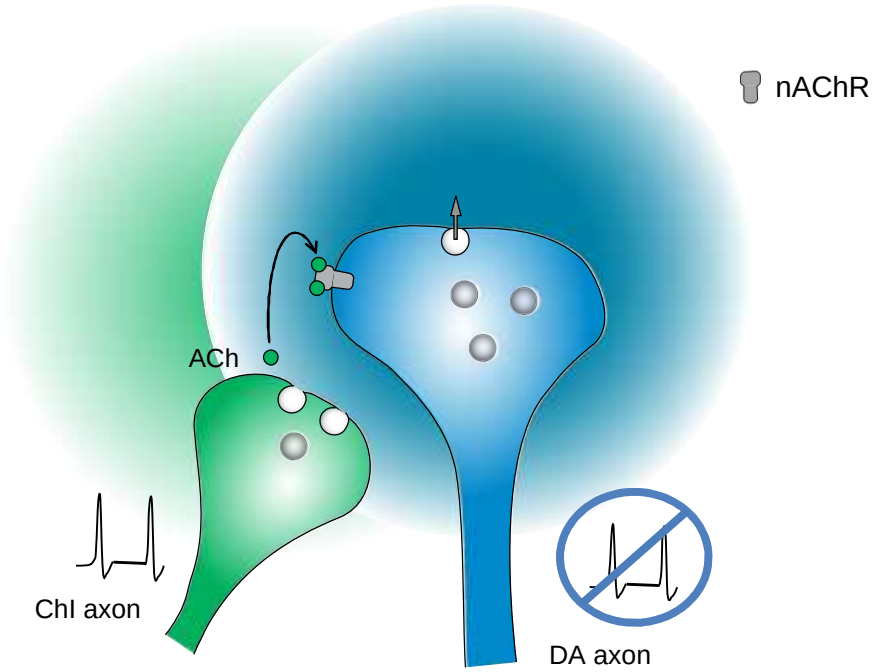


Figure 1.4 ACh directly evokes striatal DA release. ACh released by synchronous activity in ChIs can act at nAChRs on DA terminals to drive striatal DA release, even in the absence of activity in DA neurons.

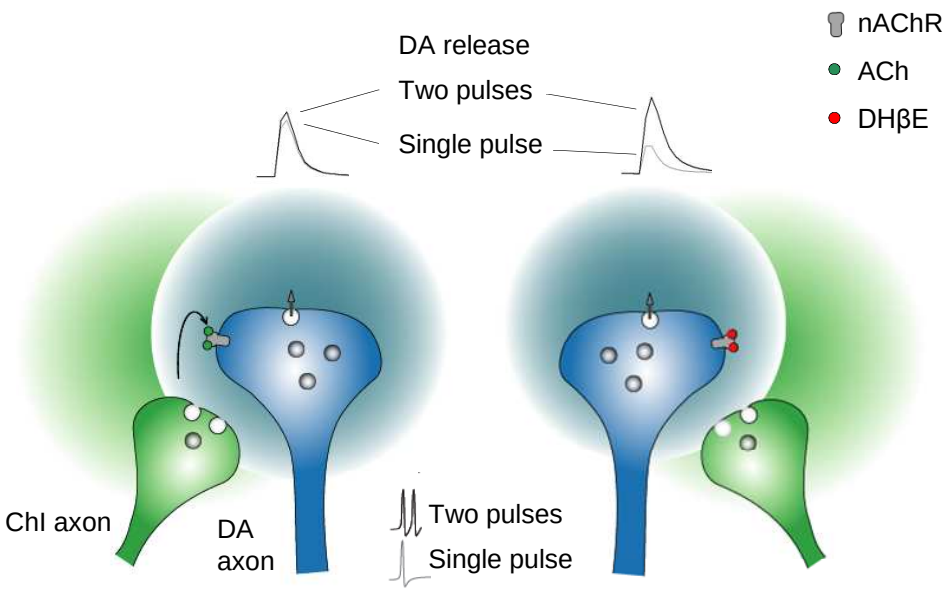


Figure 1.5 ACh modulates the short-term plasticity of striatal DA release. ACh released from ChIs acts at nAChRs on DA terminals to control the short-term plasticity of DA release. When nAChRs are active, release shows strong short-term depression so two stimulation pulses do not release much more DA than a single pulse. When nAChRs are inhibited (e.g. dihydro-β-erythroidine (DHβE) β2*-nAChR antagonist), depression is relieved and two stimulation pulses evokes significantly more DA release than a single pulse. Modified from Exley et al. 2008.

2. Methods

This chapter contains methodological background and details which pertain to this thesis as a whole. This includes a description of the model system used, discussion of FCV, discussion of methods for stimulation of DA release and characterisation of LED-evoked DA release in animals genetically engineered to express channelrhodopsin 2 (ChR2).

2.1 Murine acute brain slices as a model system for investigating DA release

We are most interested in understanding the control of DA release in relation to humans, in order to better understand human behaviour and identify novel treatment strategies for the many diseases involving DA dysfunction. However, only indirect methods of measurement, such as positron emission tomography (PET) scans to measure receptor binding density, or functional magnetic resonance imaging scans to measure neural activity in relevant brain regions, can be widely and ethically used in humans. Results from such techniques are compound measurements of many presynaptic and postsynaptic events. For example, changes in DA receptor density detected using PET scans may be due to changes in presynaptic DA release but may also be due to changes in postsynaptic receptor levels, receptor surface expression, or DA uptake. In addition, the range of pharmacological agents that can be used to investigate the control of DA release in humans is very limited for ethical reasons. Therefore, to dissect out and explore presynaptic factors controlling DA release it is necessary to use an animal model.

The animal model that has been selected in this thesis is the mouse (*Mus muculus*). This is for several reasons. Firstly, it is preferable to use a mammalian system over simpler model organisms, since the mammalian DA system more closely models the human DA system. Secondly the use of rodents is preferable to higher organisms such as primates since this reduces ethical concerns, due to the special protection afforded to primates under the Animal Scientific Procedures Act. Furthermore, the mouse has been selected due to the ease of genetic

manipulations of this species. Historically, the mouse was the first mammal to be genetically modified by targeted gene knock-out (Capecchi 1989) and one of the first mammals to have its genome fully sequenced (Mouse-Genome-Sequencing-Consortium 2002) and it remains the mammalian species most amenable to genetic manipulation. Several genetically modified models have been used in this thesis, including a genetic model of PD, synapsin III knock-out animals and Cre-recombinase expressing animals. These genetic tools have allowed me to address questions about the control of DA release which would not be possible using pharmacological agents alone. Such genetic tools are less widely available in other mammalian species, making the mouse the most appropriate model organism.

The majority of work in this thesis used the mouse strain C57Bl6/J, a common inbred laboratory mouse strain. This was chosen because C57Bl6 is the background strain for many genetically modified mouse lines, allowing for easy comparison of data between models. Additionally, C57Bl6/J mice carry the full α-syn gene, unlike some other C57Bl6 lines which carry a spontaneous deletion of this gene (Specht and Schoepfer 2001), which may affect DA release (Abeliovich *et al.* 2000).

In this thesis, acute coronal brain slices have been used as a model system. Again this is for several reasons. It is possible to directly measure DA release *in vivo* in the rodent striatum (Rice *et al.* 1985; Kuhr and Wightman 1986; Garris and Wightman 1995). This has the advantage of monitoring DA release in an intact system, with all striatal afferent and efferent connections intact. However, *in vivo* measurements carry the significant disadvantage of being inaccessible for precise experimental manipulation. For example, the direct application of known drug concentrations, manipulation of environmental factors such as temperature, or accurate anatomical placements of measuring devices, are more challenging. Also, although the intact circuitry of the *in vivo* system allows all contributions to DA release to be considered, this complexity makes it harder to dissect out individual controlling factors. For example, in the intact

brain, circuit effects such as basal ganglia efferent projections to DA neurons in the midbrain, may affect striatal DA release, in addition to presynaptic factors. Since in this thesis I am interested in investigating presynaptic factors acting in or at DA neuron terminals, the simplified system of the brain slice is preferable to *in vivo* work. In addition, the potential for the animal to suffer pain, suffering, distress or lasting harm is greater with *in vivo* studies so the use of brain slices is one method of refinement of animal research. Other possible *in vitro* systems for studying DA release include cultured DA neurons or synaptosome preparations. These systems have the advantage of being greatly simplified compared with the brain slice, and easily permit the study of presynaptic mechanisms. However, in this thesis I am also interested in how local striatal networks, particularly ACh release from ChIs, interact with presynaptic factors to control DA release. Therefore, acute brain slices have the advantage of maintaining relatively intact local striatal networks and containing DA nerve terminals *in situ*. In addition, slices have the advantage over cultured DA neurons that neuronal development has occurred in an intact physiological environment, containing all physiological growth factors, glial support and network interactions, and hence may more closely model DA neuron function in the intact brain. Therefore, in this thesis I have used *ex vivo* acute striatal brain slices to investigate the presynaptic factors controlling DA release.

Acute 300 µm thick coronal striatal slices have been used. These slices maintain local striatal networks, including interactions between MSNs, GABAergic interneurons, ChIs and DA terminals (Koos and Tepper 1999; Koós and Tepper 2002; Rice and Cragg 2004; Zhang and Sulzer 2004; Shen *et al.* 2008). Afferent striatal connections including cortico- and thalamostriatal connections are severed, but their axons remain. DA axons are also severed from their cell bodies in the midbrain but can continue to release DA for over 9 hours when stimulated (Bull *et al.* 1990).

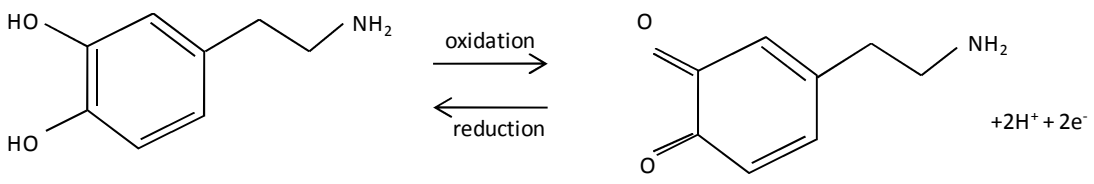
2.1.1 Methods for generating acute striatal slices

All animals were maintained and procedures carried out in accordance with UK Home Office and local guidelines. The majority of work in this thesis used adult (>35 days) male C57Bl6/J mice (Charles River).

Mice were sacrificed by cervical dislocation, the brains removed and rapidly transferred to ice-cold HEPES-based buffer solution containing in mM: 120 NaCl, 20 $NaHCO_3$, 6.7 HEPES acid, 5 KCl, 3.3 HEPES salt, 2 $CaCl_2$, 2 $MgSO_4$, 1.2 KH_2PO_4, 10 glucose, saturated with 95% O_2/5% CO_2. Coronal slices of 300 µm thickness, containing both dorsal striatum and NAc (**Fig. 2.1**), were generated, in HEPES-based buffer, using a vibratome (VT1000S or VT1200S; Leica). Slices were maintained at room temperature in HEPES-based buffer for 1 hour.

2.2 Fast-scan cyclic voltammetry

Throughout this thesis, FCV at carbon-fibre microelectrodes (CFMs) has been used to monitor evoked endogenous DA release in acute brain slices, in order to investigate factors controlling DA release. FCV is an electrochemical technique that allows the quantification of evoked endogenous catecholamine release with subsecond temporal resolution. The technique relies on the fact that catecholamines, including DA, can be easily oxidised and reduced. A triangular voltage waveform, here from -700 mV to +1300 mV and back, relative to a Ag/AgCl reference electrode, is scanned across the CFM (**Fig. 2.2**). DA oxidises to dopamine-o-quinone, and is reduced back to DA, between these potentials according to the reaction scheme below:



Dopamine

Dopamine-o-quinone

Therefore on the upward scan, any DA present at the surface of the electrode is oxidised to dopamine-o-quinone and during the reverse scan, dopamine-o-quinone is reduced back to DA. The currents generated by these redox reactions are detected by the CFM. Within the relevant range of DA concentrations measured in *ex vivo* brain slices, the size of the oxidation current is directly proportional to DA concentration. Therefore the concentration of DA present at the electrode surface can be quantified by calibrating the CFM, by applying a known concentration of exogenous DA and measuring the response of the electrode (Kelly and Wightman 1987; Bull *et al.* 1990; Stamford *et al.* 1995).

FCV has a number of advantages over other techniques used to monitor transmitter release, which make it appropriate to investigate the questions posed in this thesis. Firstly it allows the measurement of evoked endogenous transmitter release. Several other available techniques use either exogenous transmitter, for example to monitor diffusion and uptake in tissue (Kelly and Wightman 1987), or exogenous radiolabelled transmitter analogues to monitor transmitter release (Chesselet 1984). It remains unknown whether exogenous, particularly radiolabelled transmitters, are handled identically to endogenous transmitter by presynaptic terminals, for example in the way they are packaged into vesicles, or vesicles are trafficked into distinct pools. Therefore FCV has the advantage of monitoring physiologically relevant transmitter release.

Secondly FCV allows rapid subsecond measurements of DA concentration. Physiological transmitter release occurs rapidly, with time constants of release on a millisecond timescale and FCV gives a real-time readout of this rapid transmitter release. The scan rate of the triangular voltage waveform is fast (here, 800 V/s) to increase resolution between oxidation peaks. However, this high scan rate also mean scans are rapid and can therefore be applied at a high frequency. Here a scanning frequency of 8 Hz has been used. Hence the technique has a very high temporal resolution. This is in contrast to some other techniques commonly used to measure transmitter release. Microdialysis is one such technique. In microdialysis, a probe with

an internal cannula surrounded by a semi-permeable membrane, is inserted into the brain tissue. The internal cannula is slowly perfused with artificial cerebrospinal fluid (aCSF), which equilibrates with the extracellular solution of the brain by passive diffusion across the semi-permeable membrane. The collected perfusate can then be analysed using techniques such as high-performance liquid chromatography, to calculate transmitter concentrations in the extracellular space. This technique integrates data over several minutes or even longer due to the slow perfusion of aCSF required to achieve equilibrium with the extracellular solution. It therefore cannot be used to investigate short-term changes in transmitter release (Salamone 1996; Robinson and Wightman 2007). In this thesis I am particularly interested in the control of the short-term plasticity of DA release, involving changes in release over a millisecond timescale. Therefore FCV is a more appropriate method to address these questions.

Additionally FCV uses CFMs for detection of DA. These electrodes are very small; the tips of the electrodes used here have a diameter of 7-10 µm, and are implanted 100 µm into tissue. This small size minimizes tissue damage, in contrast to microdialysis probes for example, which have a diameter of more than 100 µm and can therefore cause significant tissue trauma (Bungay *et al.* 2003). In addition, the small size of the CFM gives excellent spatial resolution, allowing DA levels to be monitored in a highly localized region. This is significant here, as this thesis involves investigating the control of DA release in different striatal regions and therefore a technique with high spatial resolution is required.

Alternative electrochemical techniques, such as amperometry, can also be used to monitor transmitter release. Indeed chronoamperometry has an even higher temporal resolution, and equally good spatial resolution, compared to FCV (Michael and Wightman 1999). However, FCV is preferable for use in a striatal brain slice since, unlike amperometry, it allows species identification; that is it can be used to distinguish between DA and other amines such as 5-HT, or metabolites such as DOPAC. In the striatum, DA is likely to be the dominant oxidisable species

present in the extracellular space. However, low release of other species, such as 5-HT, and the presence of metabolites such as DOPAC, cannot be excluded. In amperometry the recording electrode is held at a given potential, and the redox current recorded at this fixed potential. Many different chemical species may oxidise at this potential and their relative contribution to the signal cannot be determined. In contrast, the triangular voltage waveform used in FCV produces a characteristic voltammogram, providing peak oxidation and reduction potentials which occur at distinct potentials for different species. In this way the waveform used in FCV assists with species identification (Stamford *et al.* 1995; Michael and Wightman 1999). Therefore, for monitoring extracellular DA concentration in a striatal slice, FCV is a more appropriate technique than amperometric methods.

Finally, FCV using CFMs is a very stable technique. With the CFMs used here there is little or no deterioration of the electrode over the course of an experiment (Stamford *et al.* 1995). Additionally, adsorption of oxidisable species to the CFM is minimised by the cyclic form of the scanning waveform and switching the electrode out of circuit between scans. Therefore, stable recordings of extracellular evoked DA release can be maintained in a single site in a single brain slice for around 9 hours, allowing multiple manipulations or drug applications at a single site (Bull *et al.* 1990).

2.2.1 Methods for producing CFMs

A carbon fibre (epoxy-covered or epoxy-free, 0.007-0.008 mm diameter, Goodfellow Cambridge Ltd, UK) was inserted into a borosilicate glass capillary tube (outer diameter 2.0 mm, inner diameter 1.16 mm, Harvard Apparatus, UK) filled with acetone for ease of insertion, to clean the carbon fibre and remove the epoxy coating if present. The acetone was then removed and a high heat applied to the capillary tube using an electrode puller (Narishige, Japan), such that a tight seal was formed around the carbon fibre. The heat setting was adjusted, such that the seal around the carbon fibre was loose enough to be visible under a 10x objective, but tight enough

to provide an adequate signal to noise ratio during recordings. The carbon fibre protruding from the seal was then cut to a length of 50-100 µm from seal to tip. To allow the electrode to be connected to the voltammeter, a piece of wire, with the end covered in silver conductance paint to form a connection with the carbon fibre, was inserted into the open end of the capillary tube, and was held in place by a small amount of superglue.

2.2.2 Methods for FCV

FCV was carried out as described previously (Exley *et al.* 2007; Threlfell *et al.* 2010). Acute striatal brain slices, generated as described above, were transferred into the recording chamber and superfused at approximately 1.5 ml/min, with a bicarbonate-based buffer aCSF solution containing, in mM: 124.3 NaCl, 26 $NaHCO_3$, 3.8 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.2 KH_2PO_4, 10 glucose, saturated with 95%O_2/5%CO_2, at 31-33^{o}C. A CFM was inserted 100 µm into the tissue. Slices were left to equilibrate and the CFM to charge for 30-60 minutes prior to recordings. Throughout this thesis I have been interested in investigating the control of DA release between two striatal regions, dorsal striatum and NAcC, which have different behavioural functions as discussed in chapter 1.1. Experiments using a single recording site have therefore been carried out either in the dorsolateral quadrant of the striatum or in the NAcC. These sites have been referred to throughout this thesis as dlCPu or NAcC respectively (**Fig. 2.1**). A triangular voltage waveform (-700 to + 1300 mV and back, vs Ag/AgCl reference electrode, scan rate 800 V/s) was scanned across the CFM using a Millar voltammeter (**Fig. 2.2**). A sampling frequency of 8 Hz was used. The species being detected was confirmed as DA by comparison of the voltammogram (**Fig. 2.2**) with that produced by the application of exogenous DA in aCSF to the electrode during post-experiment calibration. Currents at the peak oxidation potential were measured from the baseline of the voltammogram. After each experiment the CFM was calibrated. 2 µM DA in aCSF, a concentration within the range of experimental measurements, was rapidly perfused over the CFM and the current at the peak oxidation potential was measured. Calibration solutions were

made up immediately before use from stock solutions of 2.5 mM DA in $HClO_4$ stored at 4^{o}C. The range of electrode sensitivity was approximately 6.0-25.0 nA/µM. Since the size of the oxidation current is directly proportional to DA concentration within the range of experimental measurements (**Fig. 2.3**), this calibration value allowed the conversion of recorded oxidation currents to DA concentrations. These concentrations were plotted against time to provide profiles of evoked extracellular DA concentration ($[DA]_o$) against time.

Data were acquired and analysed using Axoscope 10.2 (Molecular Devices) or Strathclyde Whole Cell Program (University of Strathclyde, Glasgow, UK) and locally written programs. Significance was considered $p < 0.05$. The following symbols have been used to indicate statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

2.3 Evoking DA release

As described above, in coronal striatal slices DA axons are severed from their cell bodies. Correspondingly, we do not detect spontaneous DA release in our slices. Therefore, the slices are stimulated to evoke DA release. In this thesis two methods of stimulation have been used. The majority of experiments use electrical stimulation to evoke release. In some experiments an optogenetic approach has been used, using light to stimulate transmitter release in slices from animals genetically modified to express light-activatable ion channels. These two techniques are described further below.

2.3.1 Electrical stimulation

Electrical stimulation uses an electrical stimulator placed on the surface of the slice to pass a current over the surface of the slice, generating action potentials in the cell bodies and axons directly under, and locally to, the stimulator. This stimulation activates all neuronal types present in the vicinity of the stimulator (although they may have different stimulation thresholds) and

hence elicits the release of multiple transmitters, in addition to the DA release that is monitored using FCV. It has the advantage that, unlike optogenetic stimulation, it can be used in WT mice, which may be a more physiologically relevant model than the genetically modified animals used in optogenetic experiments. In addition, it is a technique that has been used for many years and hence is well characterised and understood (Stamford *et al.* 1995).

2.3.1.1 Methods for Electrical stimulation

A surface bipolar concentric Pt/Ir electrode (25 µm diameter, FHC) was placed locally in the striatum on the surface of the tissue, approximately 100 µm from the CFM. Electrical pulses of 200 µs duration, 0.6 mA amplitude (a perimaximal current, that is the minimum current that evokes maximum DA release with a single 200 µm pulse) were applied to evoke DA release, unless described otherwise. Stimulations were applied at 2.5 minute intervals unless described otherwise. $[DA]_o$ evoked by subsecond stimulations, with 2.5 min intervals between stimulations, usually declines over the initial 4-5 stimulations in a release site, after which release becomes sustainable and reproducible for up to approximately 8 hours. Unless described otherwise, data were only included for analysis after $[DA]_o$ was stable and reproducible.

2.3.2 Optical stimulation

In some experiments an optogenetic stimulation approach has been used. This approach uses light to control the activity of genetically-defined groups of neurons, via exogenously expressed proteins called opsins. Opsins bind to the chromophore retinal, making them light sensitive. Type I opsins, expressed endogenously in some prokaryotes, archaea and fungi, directly transduce light energy into changes in membrane potential by functioning as light-activatable ion-pumps or channels in the cell membrane. In contrast to mammalian opsins, which couple light-induced changes in the chromophore retinal to intracellular signalling pathways, type I opsins are single component systems, that is they function as both the light sensor and effector. Therefore transduction is very rapid, much more rapid than that of mammalian rhodopsins. In 2003 Nagel

et al. showed that a type I opsin could be expressed and function in heterologous systems, including mammalian cells. Since then, using different genetic tools, opsins have been expressed in many different cell types, particularly different neuronal populations, in mammals (Boyden *et al.* 2005; Nagel *et al.* 2005; Bi *et al.* 2006; Tsai *et al.* 2009).The combination of this rapid phototransduction with various genetic tools allows type I opsins to be used to precisely control the activity of specific neuronal populations using light stimulation.

In some areas of my thesis I have chosen to use an optogenetic approach to investigate the presynaptic control of DA release. This is primarily because, as discussed above, electrical stimulation activates all neuronal populations in the locality of the stimulating electrode. In contrast, optical stimulation will only activate neurons in which type I opsins are expressed, allowing for the selective activation of a genetically defined neuronal population, here either DA neurons or ChIs. By expressing opsins selectively in DA neurons, this approach has allowed me to investigate the control of DA release in the absence of confounding network effects, without the use of a barrage of pharmacological agents. Alternatively, by expressing opsins selectively in ChIs, I have been able to investigate the role of ChIs in controlling DA release, in the absence of activity in DA neurons or other confounding network effects. Therefore optogenetics has allowed me to tease apart the contributions of different neuronal populations to the control of DA release.

In addition, optical stimulation is less invasive than electrical stimulation. As described above, the electrical stimulator is placed on the surface of the tissue. While the tissue damage that this placement, or electrical stimulation, causes is minimal, optical stimulation further reduces any such damage.

2.3.2.1 Choice of opsin

There are three classes of type I opsin which transduce light to ion movement across the cell membrane: bacteriorhodopsins, which are light-activated proton pumps, channelrhodopsins, light-activated cation channels, and halorhodopsins, light-activated chloride pumps. All three

classes can be expressed in mammalian neurons and used to drive changes in neuronal activity *in vitro* or *in vivo* (Yizhar *et al.* 2011). In this thesis I have used a channelrhodopsin to activate neurons and evoke DA release. When activated by light both halorhodopsins and bacteriorhodopsins hyperpolarise and silence neurons. Since DA axons in acute striatal slices are already quiescent, these opsins are not useful tools to investigate the control of DA release in this preparation.

The first channelrhodopsin used to control mammalian neuronal activity was ChR2, when it was shown that light activation of ChR2 could be used to reliably drive spikes in cultured neurons (Boyden *et al.* 2005). ChR2 has relatively rapid opening and desensitisation kinetics, with a current rise time of ~200 μs, and a desensitisation time constant of ~20 ms at pH7 (Nagel *et al.* 2003). This relatively rapid photocycle allows ChR2 to reliably follow frequencies of stimulation up to 20-40 Hz (Boyden *et al.* 2005; Tsai *et al.* 2009). ChR2 has a low single channel conductance, estimated to be in the femtoampere range (Nagel *et al.* 2003), but can be expressed and tolerated at relatively high expression levels in mammalian neurons, generating whole-cell inward currents of approximately 200-500 pA (Boyden *et al.* 2005). Its action spectrum peaks at 460-470 nm, so it is maximally activated by blue light. To date, ChR2 is the best characterized and most widely used channelrhodopsin.

In recent years, many channelrhodopsin variants with different characteristics have become available. Some variants have been generated with increased single channel conductance, such as the H134R mutant (Nagel *et al.* 2005), and others have increased kinetics of channel closure and recovery from desensitisation and inactivation, and so can follow higher frequencies of stimulation. For example ChETA channelrhodopsins, which have an E123T/A mutation, can respond to frequencies of light stimulation of up to 200 Hz (Gunaydin *et al.* 2010). Such mutations involve trade-offs in the characteristics of the opsin. For example the H134R mutation increases single channel conductance but as a result slows closure kinetics, and conversely ChETA

mutants have increased kinetics but decreased single channel conductance. Hence there is now a large array of channelrhodopsin tools available, with distinct advantages and disadvantages, so the selection of an appropriate channelrhodopsin to address the questions posed is important.

In this thesis the classic channelrhodopsin, ChR2, has been used. This is because currents generated by ChR2 are sufficient to reliably drive DA neuron activation at frequencies up to 20-30 Hz (Tsai *et al.* 2009; Bass *et al.* 2010), which is representative of DA neuron firing frequencies seen *in vivo* (Grace and Bunney 1984). ChETA mutants would allow activation of axons at frequencies across the entire range seen *in vivo*, and at frequencies of up to 100 Hz, which can be a useful tool for investigating presynaptic mechanisms (Rice and Cragg 2004; Threlfell *et al.* 2010). However, preliminary experiments in our lab found that the lower single channel conductance of ChETA channelrhodopsin meant it did not reliably generate spiking activity and DA release when expressed in DA neurons (unpublished observations).

2.3.2.2 Channelrhodopsin expression

There are several different available methods for inducing ChR2 expression in mice. One approach is to use genetically modified mice in which ChR2 is incorporated into the genome under the control of a cell type-specific promoter (Arenkiel *et al.* 2007; Zhao *et al.* 2011). These mouse lines have stable and reliable ChR2 expression. However, they express ChR2 in all neurons of a particular type, and so do not provide any regional specificity. A more common approach is to use viruses to deliver the ChR2 gene. This delivers a high gene copy number and hence results in high expression levels. It also allows anatomical control of expression, via restricted injection sites. However, few cell type-specific promoters are small enough to be easily packaged into a virus. Therefore genetic targeting can be difficult using viral delivery. One common approach to achieve high expression levels but also precise genetic targeting, which has been used in this thesis, is to combine viral delivery of a floxed gene encoding ChR2, with Cre-recombinase-expressing mouse driver lines. In this method the ChR2 gene is packaged into a virus in an

inverted orientation, preventing its expression, and doubly flanked by loxP sites (**Fig. 2.4**). The DNA recombinase enzyme, Cre-recombinase, can homologously recombine the DNA at these loxP sites, inverting the ChR2 gene and allowing it to be expressed. The virus is delivered into animals genetically engineered to express Cre-recombinase under a cell-type specific promoter. Expression of ChR2 is then driven solely in the neuronal populations expressing Cre-recombinase. Here, mouse lines expressing Cre-recombinase under control of the DAT promoter (DAT^{Cre}) and ChAT promoter ($ChAT^{Cre}$) have been used to induce ChR2 expression in DA neurons and ChIs respectively. In this way, high expression levels, genetic specificity of expression and anatomical specificity can be combined.

2.3.2.3 Virus production

The Cre-inducible recombinant AAV vector construct containing the ChR2 gene inframe with the coding sequence for enhanced yellow fluorescent protein (eYFP) (**Fig. 2.4**) was a gift from Prof Karl Deisseroth (Stanford University). There are many possible viruses which can be used for ChR2 gene delivery. Here, an adeno-associated virus (AAV), AAV5, was selected, since AAVs allow infection of a large brain region and hence ensure high expression levels throughout the region of interest (Yizhar *et al.* 2011). The vector constructs were serotyped with AAV5 coat proteins and packaged to a final viral concentration of 10e12 particles/ml by the viral vector core at the University of North Carolina.

2.3.2.4 Methods for stereotaxic surgery for AAV5-ChR2 delivery

All animals were maintained and procedures carried out in accordance with UK Home Office and local guidelines. Adult heterozygote DAT^{Cre} or $ChAT^{Cre}$ male mice ($DAT^{Cre/+}$, $ChAT^{Cre/+}$), on a C57Bl6/J background (bred from stock from Jackson laboratories, B6.SJL-*Slc6a3*$^{tm1.1(cre)Bkmn}$/J, stock # 006660; B6.129S6-*Chat*$^{tm1(cre)Lowl}$/J, stock # 006410) were used. Mice were anaesthetized using isoflurane and placed in a stereotaxic frame. 7.5% marcaine was applied as a local anaesthetic and craniotomy was performed. Injections of either 0.2 µl fluorescent beads and 0.4-

0.8 µl virus or 0.5-1 µl virus alone were given using a 33 gauge Hamilton syringe, at a rate of approximately 0.2 µl/min. In $DAT^{Cre/+}$ mice injections were made either unilaterally or bilaterally in the midbrain, in either the VTA (co-ordinates from Bregma in mm: anterior-posterior (AP) -3.1, medial-lateral (ML) ±0.5, dorsal-ventral (DV) -4.4) or the SNc (AP -3.5, ML ±1.2, DV -4.0). In $ChAT^{Cre/+}$mice injections were made either unilaterally or bilaterally in either the dorsal CPu (AP 1.0, ML ±1.75, DV -2.2) or NAcC (AP 1.0, ML±1.0, DV -4.0). When bilateral injections were given, different regions in each hemisphere were targeted. After each injection the needle was left in place for 10 minutes and retracted slowly. The wound was then sutured using Ethilon monocryl non-absorbable suture. 0.5-0.7 ml 0.9% sterile saline was injected subcutaneously to replace lost fluids. Animals were maintained for at least 10 days following surgery to allow time for virus infection and ChR2 expression.

2.3.2.5 Methods for optical stimulation

Two different methods of optical stimulation were used. The first was a laser system, using a 473 nm diode laser (DL-473, Rapp Optoelectronic) coupled to the microscope via an optic fibre (200 µm multimode, numerical aperture 0.22). This illuminated a 15/60 µm diameter spot (10/40x water-immersion objectives). The second was an LED system, using a 470 nm LED (OptoLED, Cairn Research), which illuminated the full-field of view, 2.2 mm (10x water-immersion objective, field number of eyepiece 22). For both systems, the light source was driven by a 2.0 ms TTL pulse, with a perimaximal light intensity, that is the minimum intensity that evokes maximum DA release with a single 2.0 ms pulse. As with electrical stimulation, stimulations were applied at 2.5 min intervals, allowing reproducible and sustainable release.

2.4 Characterisation of LED system for optical stimulation

This optogenetic approach for evoking DA release has been established and characterised in our lab using the diode laser system. However, DA release evoked by the LED system has not been

characterised. Therefore, before beginning experiments using this system, it was necessary to characterise LED-evoked DA release with respect to LED stimulation parameters, and to establish appropriate stimulation protocols. Firstly, an 8.0 mW/mm^2 light pulse, pulse width 2.0 ms, was used to evoke release in striatal slices from both DAT$^{Cre/+}$ and ChAT$^{Cre/+}$ mice, expressing ChR2. The voltammograms were compared with that produced by the application of exogenous DA in aCSF to the electrode during post-experiment calibration, and the species detected was hence confirmed as DA (**Figs. 2.5, 2.6**). NE produces an identical voltammogram, but given that transmitter release in this striatal region (Palij *et al.* 1990) using similar stimulation parameters (Threlfell *et al.* 2012) has been previously confirmed as exclusively DA, a contribution from NE is unlikely.

Secondly, the relationship between LED light intensity and $[DA]_o$ was explored in slices from both DAT$^{Cre/+}$ and ChAT$^{Cre/+}$ animals expressing ChR2, in both dlCPu and NAcC. As expected, and similarly to the relationship between both electrical stimulation current and $[DA]_o$ (Cragg 2003; Exley *et al.* 2007), and laser stimulation and $[DA]_o$ (Threlfell *et al.* 2012), in NAcC in DAT$^{Cre/+}$ mice and in both regions in ChAT$^{Cre/+}$ mice, there was a positive relationship between light intensity and $[DA]_o$ which reached an asymptote at higher intensities (**Figs. 2.7, 2.8**). However in dlCPu in DAT$^{Cre/+}$ mice, the positive relationship did not reach an asymptote (**Fig. 2.7**). This is most likely due to a lower level of ChR2 expression in dlCPu in DAT$^{Cre/+}$ mice, most likely because of relative difficulty targeting the SNc with stereotaxic injections and reduced viral spread in this region. However, these results demonstrate that LED stimulation of ChR2 expressing neurons in slices from DAT$^{Cre/+}$ or ChAT$^{Cre/+}$ animals is not supramaximal and shares characteristics with electrical stimulation. The light intensity at which an asymptote was reached, or the perimaximal intensity, was found to vary between animals, mostly likely due to variability in ChR2 expression. Therefore, in further optogenetic experiments in this thesis the perimaximal light intensity was determined at the beginning of each experiment and used throughout the experiment. In some

cases where a perimaximal intensity could not be determined in dlCPu of $DAT^{Cre/+}$ mice, the highest possible intensity was used.

The relationship between pulse width and $[DA]_o$ was also explored in both mouse lines, in both dlCPu and NAcC (**Figs. 2.9, 2.10**). There was a positive relationship between pulse width and $[DA]_o$ at low pulse widths, that reached an asymptote at longer widths, in both mouse lines, in both regions. In $DAT^{Cre/+}$ mice, the relationship between pulse width and $[DA]_o$ appeared to reach an asymptote at lower pulse widths in NAcC than in dlCPu. This is most likely due to differences in ChR2 expression levels in different regions. In $ChAT^{Cre/+}$ mice, the relationship between pulse width and $[DA]_o$ was similar between regions. Throughout optogenetic experiments using the LED system a pulse width of 2 ms has been used, since this is an approximately perimaximal pulse width (**Figs. 2.9, 2.10**), and hence maximizes DA signals without stimulating terminals supramaximally, and has been used previously using the laser system (Threlfell *et al.* 2012).

Finally, the frequency response of $[DA]_o$ was explored in $DAT^{Cre/+}$ mice using the LED system. This was firstly to confirm that light stimulation using the LED system is capable of driving trains of action potentials in DA axons, similarly to electrical and laser stimulation. Additionally, it is known that stimulation current may subtly alter the short-term plasticity and hence frequency response of DA release (see chapter 3). Since in dlCPu in some $DAT^{Cre/+}$ animals a perimaximal intensity could not be reached, it was important to explore if the frequency response of DA release would be affected by this. Trains of 2.0 ms LED pulses, across the range of stimulation frequencies used with electrical stimulation (4p, 5-100 Hz) were applied to acute striatal slices from $DAT^{Cre/+}$ mice transfected with AAV5-ChR2. The ratio between peak evoked $[DA]_o$ by train vs. single pulse stimulation (4p:1p) was calculated to explore the frequency sensitivity of release. This relationship was explored at two different light intensities, 6.6 and 8.0 mW/mm^2.

In both regions, and as expected in the absence of the confounding effects of ACh, there was a positive relationship between stimulation frequency and 4p:1p in both regions, at frequencies up

to 25 Hz (**Fig 2.11**). At frequencies higher than 25 Hz, 4p:1p decreased (**Fig. 2.11**). When electrical stimulation is used to evoke DA release when nAChRs are not active, the positive relationship between 4p:1p and stimulation frequency is maintained at frequencies up to 100 Hz (Rice and Cragg 2004; Threlfell *et al.* 2010). This suggests that, similarly to with the laser system for optogenetic stimulation (unpublished observations), light stimulation of DA axons expressing ChR2 using the LED system is not capable of reliably driving action potentials at frequencies above 25 Hz. This is consistent with previous reports of decreased reliability of ChR2 in following trains of light pulses above 20-30 Hz (Boyden *et al.* 2005; Witten *et al.* 2010), due to the intrinsic kinetics of ChR2. Therefore, in optogenetic experiments in this thesis, only frequencies of stimulation up to 25 Hz have been used.

The frequency response of $[DA]_o$ was unchanged by light intensity in both regions (**Fig. 2.11**). This suggests that the fact that the intensity used in dlCPu in $DAT^{Cre/+}$ mice was sometimes necessarily below maximal is unlikely to affect the short-term plasticity of DA release.

2.5 Immunohistochemistry

Immunofluorescence was used to confirm the cell-type specific expression of ChR2. ChR2 was tagged with the fluorescent label eYFP (see **Fig. 2.4**) so ChR2 expressing neurons were fluorescently labelled. Anti-TH staining was used to identify catecholaminergic neurons in slices from $DAT^{Cre/+}$ mice and anti-ChAT staining was used to indentify cholinergic neurons in slices from $ChAT^{Cre/+}$ mice.

Following FCV recordings, slices were fixed in neutral-buffered 10% formalin solution (Sigma Aldrich) or in 4% paraformaldehyde dissolved in phosphate-buffered saline (PBS) containing 0.2% picric acid, and stored at +4^{o}C for at least 3 days. Slices were re-sectioned in PBS using a vibratome (VT1000S, Leica) to a thickness of 50 µm and stored in PBS at -4^{o}C overnight. Sections were washed in PBS.

For TH immunohistochemistry, slices were incubated in PBS containing 0.5% Triton X-100 (PBS-Tx), 10% normal goat serum and 10% fetal bovine serum for 30 minutes. Slices were then incubated in PBS-Tx, 1% normal goat serum, 1% fetal bovine serum and 1:2000 primary antibody (rabbit anti-TH; Sigma Aldrich) at 4^{o}C overnight. Slices were then washed in PBS before incubation in PBS-Tx, 1% normal goat serum, 1% fetal bovine serum and 1:1000 secondary antibody (DyLight 594 goat anti-rabbit; Jackson) for 2 hours at room temperature.

For ChAT immunohistochemistry, slices were incubated in PBS-Tx and 10% donkey serum for 30 minutes. Slices were then incubated in PBS-Tx, 3% donkey serum and 1:200 primary antibody (goat anti-ChAT; Millipore) at 4^{o}C overnight. Slices were then washed in PBS before incubation in PBS-Tx, 3% donkey serum and 1:1000 secondary antibody (Alexa Fluor 568 donkey anti-goat; Invitrogen), for 2 hours at room temperature.

Slices were washed in PBS and mounted on gelled slides with Vectashield mounting medium (Vector Labs) and imaged using an Olympus BX51WI microscope.

Co-labelling with ChR2-eYFP and immunofluorescence was examined. All ChR2 expressing cells were found to be labelled positively for TH or ChAT in DAT$^{Cre/+}$ or ChAT$^{Cre/+}$ slices respectively, confirming that ChR2 was present only in catecholaminergic neurons in DAT$^{Cre/+}$ mice and only in cholinergic neurons in ChAT$^{Cre/+}$ mice. **Figs. 2.12** and **2.13** show representative co-labelled cells from DAT$^{Cre/+}$ and ChAT$^{Cre/+}$ mice respectively.

2.6 Conclusion

FCV is a powerful technique for monitoring changes in extracellular catecholamine concentration with excellent spatial and temporal resolution. In the following chapters FCV has been used to investigate the presynaptic and network control of striatal DA release and the short-term plasticity of release, in dorsal and ventral striatal regions. DA release has been evoked using

either electrical stimulation or an optogenetic approach, to tease apart the role of different neuronal populations in controlling striatal DA release. The methods described above apply to the majority of experiments in this thesis. Additional methodological details, including experimental designs, statistical analyses and drug information, are given within each chapter where relevant.

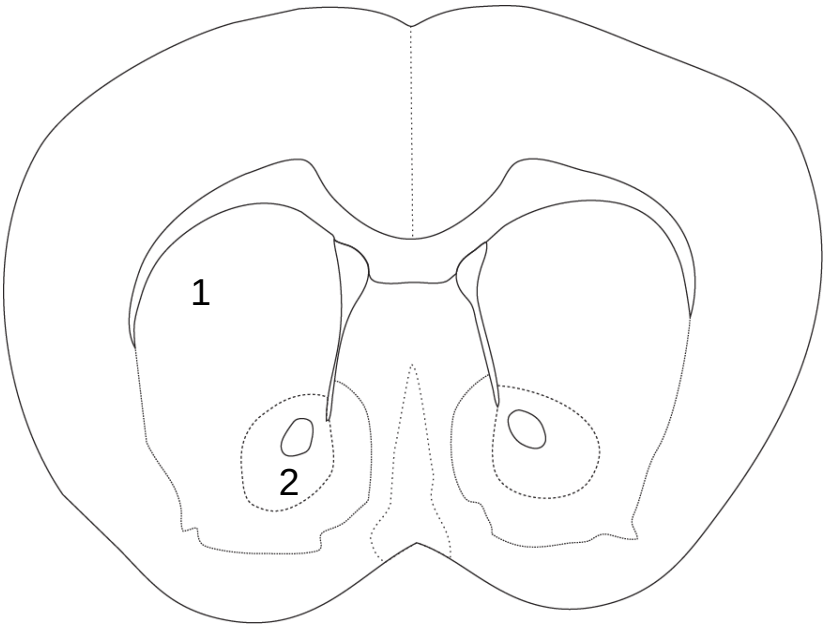


Figure 2.1. Diagram of coronal striatal slice with representative recording sites in dorsal striatum and NAc. Acute coronal striatal slices were generated containing both dorsal striatum and NAc. Unless described otherwise, recording sites were used either in the dorsolateral quadrant of the dorsal striatum (dlCPu, 1) or in the nucleus accumbens core (NAcC, 2).

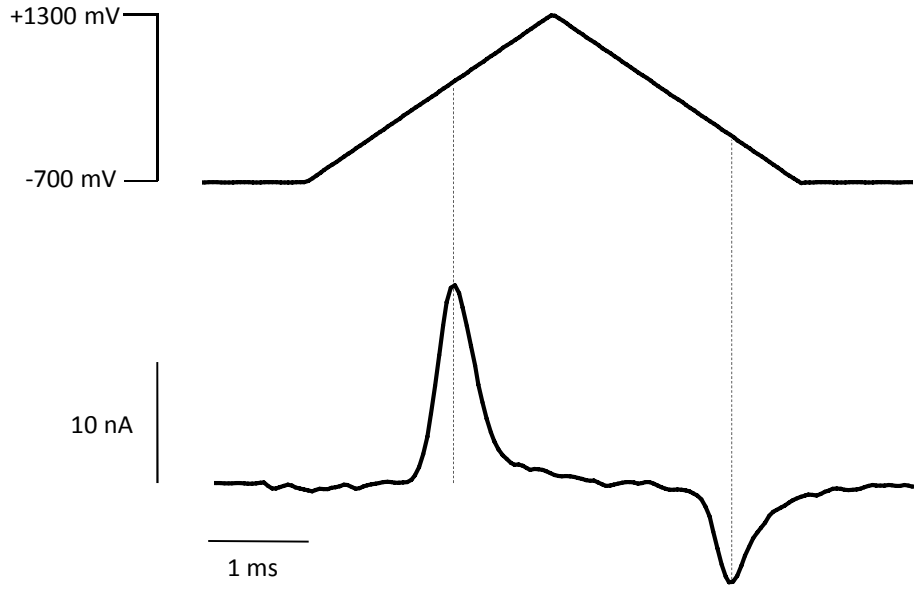


Figure 2.2. Triangular voltage waveform and representative DA voltammogram. Voltage waveform scan from -700 mV to +1300 mV relative to Ag/AgCl reference electrode. Representative voltammogram of striatal DA evoked by electrical stimulation, showing characteristic oxidation and reduction peaks at +600 mV and -200-250 mV respectively.

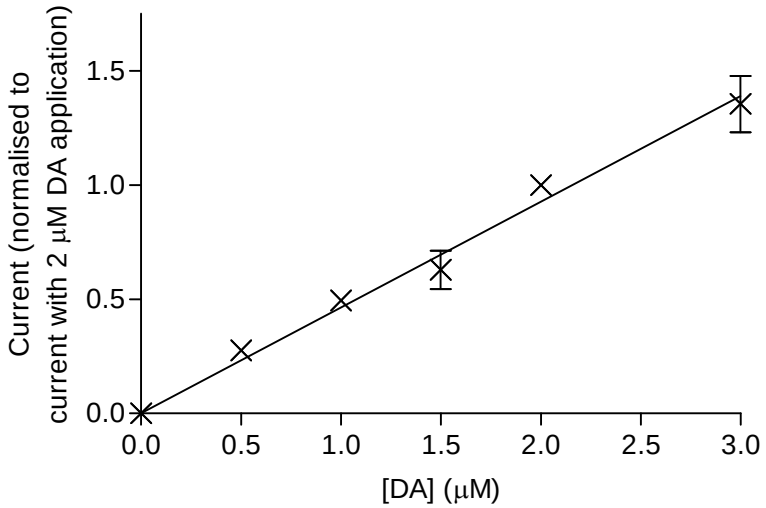


Figure 2.3. Currents recorded at CFMs are directly proportional to [DA] within the relevant experimental range. Mean peak oxidation currents ±SEM recorded at CFMs, normalised to the current produced by application of 2 µM DA, against concentration of applied DA, across a range of DA concentrations reflecting those seen during electrical stimulation of *ex vivo* striatal slices. Currents are directly proportional to applied [DA] within this range (R^2=*0.99*; *n = 3*).

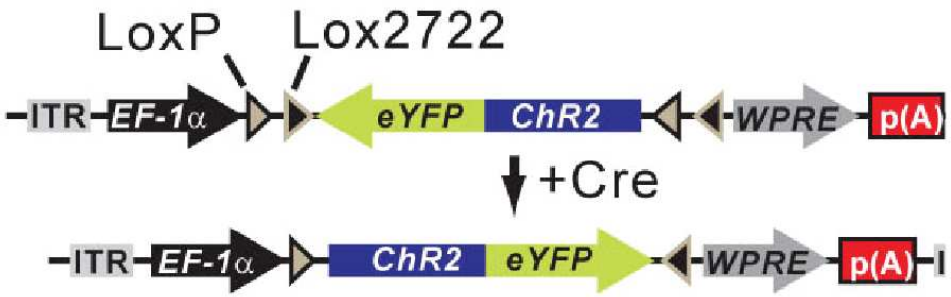


Figure 2.4. DNA construct used to transfect Cre-recombinase expressing mouse lines. The gene encoding eYFP-tagged ChR2 (ChR2-eYFP) is doubly flanked by LoxP sites in an inverted configuration to minimise leak expression. When inverted by Cre-recombinase, in Cre-expressing mouse driver lines, ChR2 expression is driven by the strong ubiquitous promoter EF-1α (Figure from Threlfell et al. 2012).

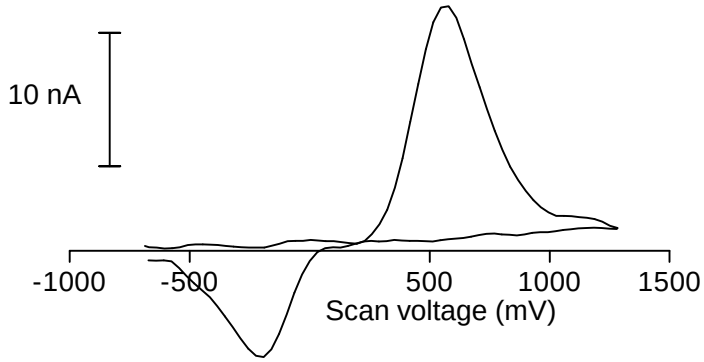


Figure 2.5. Cyclic voltammogram of $[DA]_o$ evoked by LED stimulation in slice from a $DAT^{Cre/+}$ mouse, transfected with AAV5-ChR2. Representative voltammogram shows characteristic oxidation and reduction peaks at +600-650 and -200-250 mV respectively.

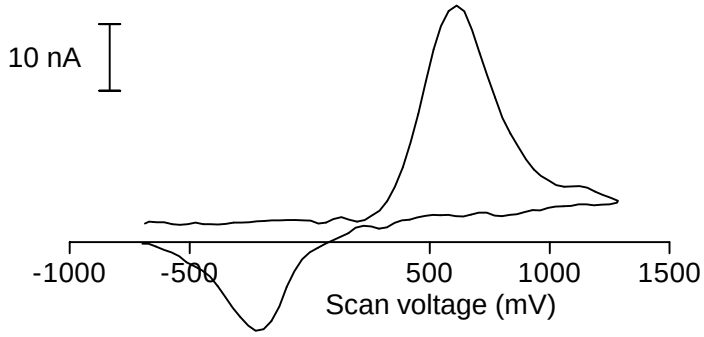


Figure 2.6. Cyclic voltammogram of $[DA]_o$ evoked by LED stimulation in a slice from a $ChAT^{Cre/+}$ mouse, transfected with AAV5-ChR2. Representative voltammogram shows characteristic oxidation and reduction peaks at +600-650 and -200-250 mV respectively.

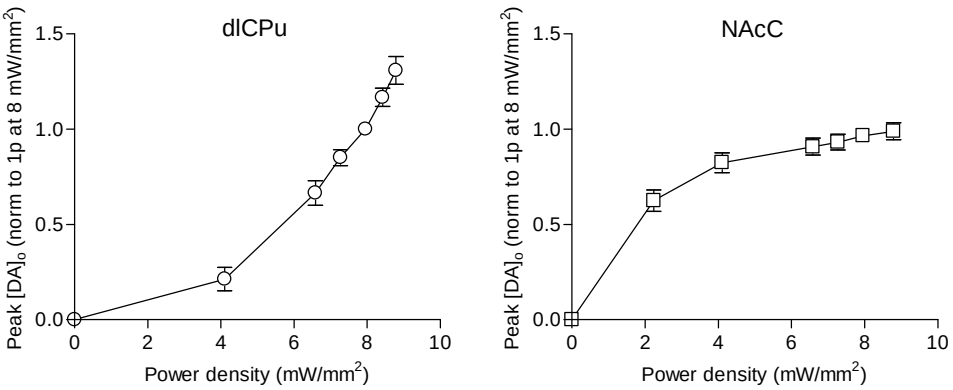


Figure 2.7. Relationship between LED intensity and $[DA]_o$ in $DAT^{Cre/+}$ mice. Mean peak $[DA]_o$±SEM evoked by single LED pulse, against light intensity, in dlCPu, left, and NAcC, right, in slices from $DAT^{Cre/+}$ mice transfected with AAV5-ChR2. In both dlCPu and NAcC there was a positive relationship between light intensity and $[DA]_o$. In NAcC this relationship reached an asymptote at higher intensities, but in dlCPu it did not.

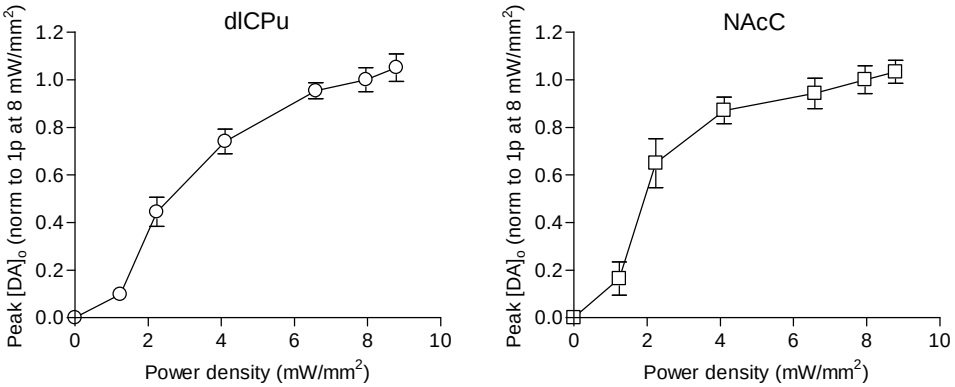


Figure 2.8. Relationship between LED intensity and $[DA]_o$ in $ChAT^{Cre/+}$ mice. Mean peak $[DA]_o$±SEM evoked by single LED pulse, against light intensity in dlCPu, left, and NAcC, right, in slices from $ChAT^{Cre/+}$ mice transfected with AAV5-ChR2. In both dlCPu and NACc there was a positive relationship between intensity and $[DA]_o$ at low intensities, that reached an asymptote at higher intensities.

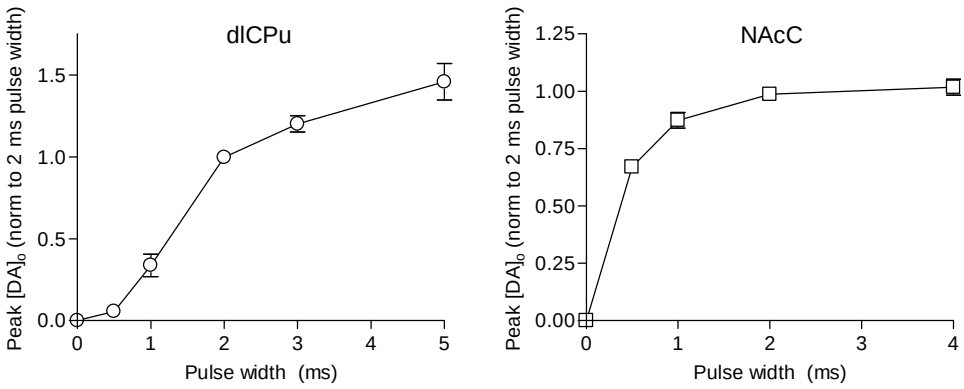


Figure 2.9. Relationship between LED pulse width and $[DA]_o$ in $DAT^{Cre/+}$ mice. Mean peak $[DA]_o$±SEM evoked by single LED pulse, against pulse width in dlCPu, left, and NAcC, right, in slices from $DAT^{Cre/+}$ mice transfected with AAV5-ChR2. In both dlCPu and NACc there was a positive relationship between pulse width and $[DA]_o$ at short pulse widths, that began to reach an asymptote at longer pulse widths.

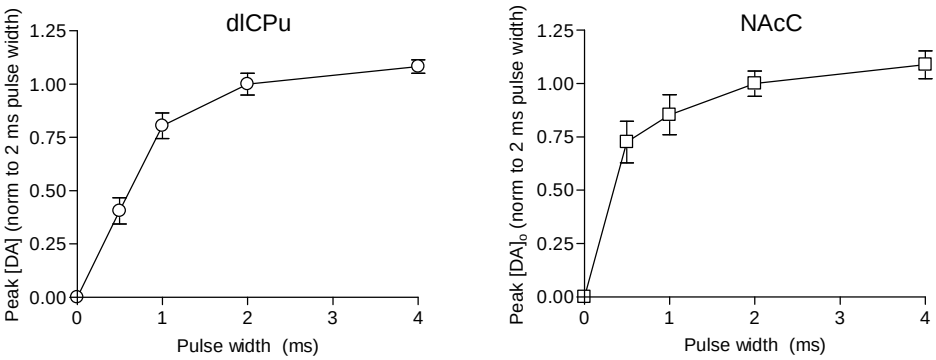


Figure 2.10. Relationship between LED pulse width and $[DA]_o$ in $ChAT^{Cre/+}$ mice. Mean peak $[DA]_o$±SEM evoked by a single LED pulse, against pulse width in dlCPu, left, and NAcC, right, in slices from $ChAT^{Cre/+}$ mice transfected with AAV5-ChR2. In both dlCPu and NAcC there was a positive relationship between pulse width and $[DA]_o$ at short pulse widths, that reached an asymptote at longer pulse widths.

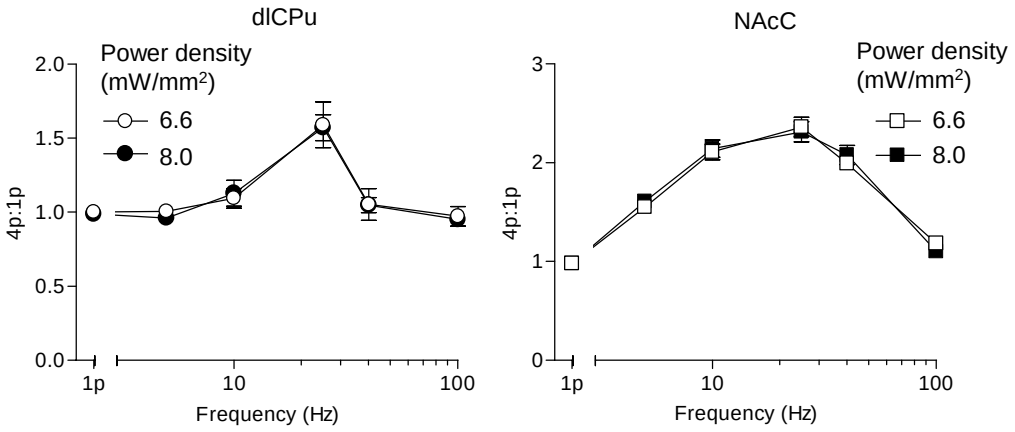


Figure 2.11. Frequency response of $[DA]_o$ evoked by LED stimulation in $DAT^{Cre/+}$ mice at two different LED intensities. Mean ratio of $[DA]_o$ evoked by train vs. single pulse stimulation (4p:1p)±SEM versus frequency of burst stimulation in dlCPu, left, and NAcC, right, using 2 different light intensities. In both dlCPu and NACc, there was a positive relationship between 4p:1p at frequencies up to 25 Hz. At frequencies above 25 Hz, 4p:1p was reduced. There was no difference in frequency response with different light intensities, in either region (Two-way ANOVA, dlCPu: main effect of light intensity $p > 0.05$ $F_{1,96}$=0.07, main effect of frequency $p < 0.001$ $F_{5,96}$=18.56, interaction $p > 0.05$, $F_{5,96}$=0.06; $n = 3$; NAcC: main effect of light intensity $p > 0.05$ $F_{1,96}$=0.03, main effect of frequency $p < 0.001$ $F_{5,96}$=131.28, interaction $p > 0.05$, $F_{5,96}$=0.43; $n = 3$).

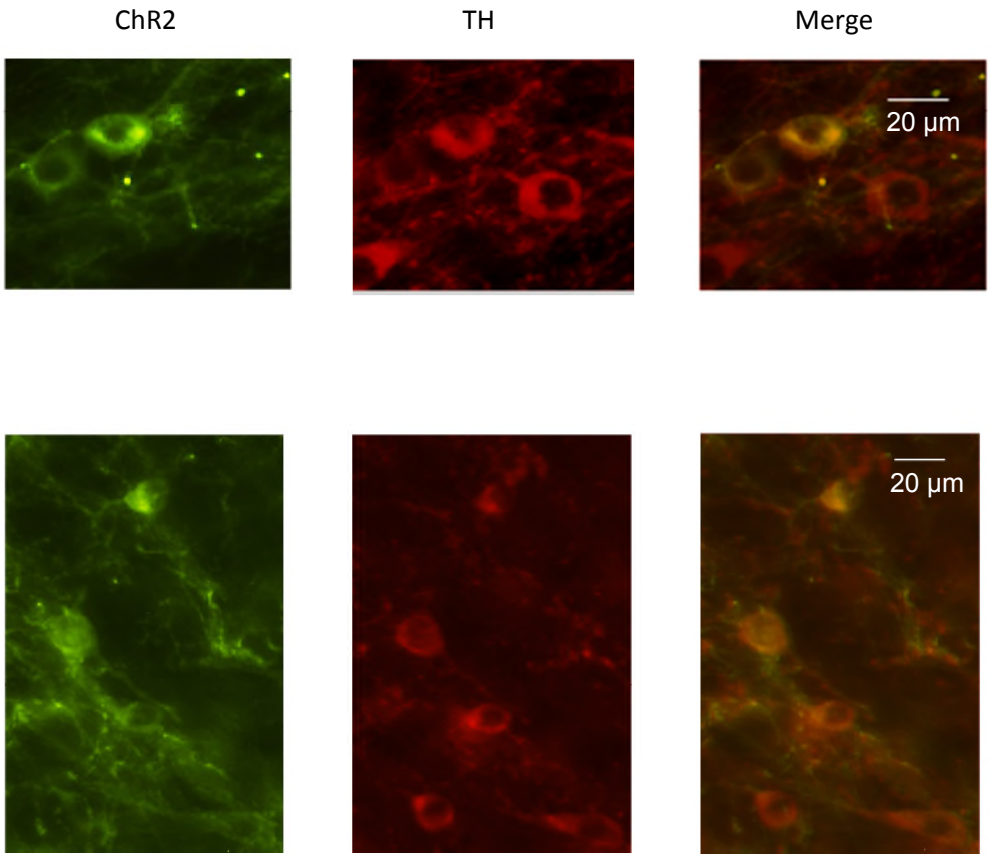


Figure 2.12. Immunofluorescent histochemistry confirms that ChR2 is expressed selectively in catecholaminergic neurons in the midbrain in DAT$^{Cre/+}$ mice. Two representative examples (top and bottom) of DA neurons in the midbrain of DAT$^{Cre/+}$ animals transfected with AAV5-ChR2. ChR2-eYFP (green, left) shows colocalisation with TH (red, middle), see merge (right).

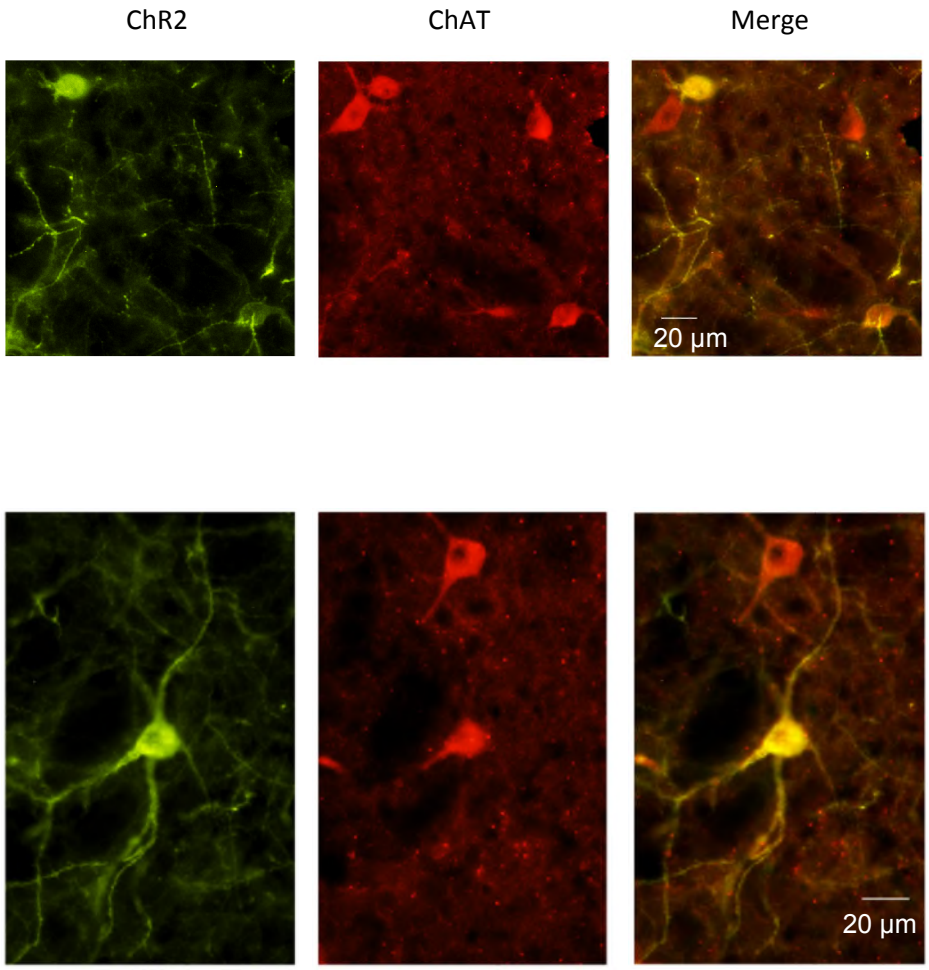


Figure 2.13. Immunofluorescent histochemistry confirms that ChR2 is expressed selectively in ChIs in the striatum in ChAT$^{Cre/+}$mice. Two representative examples (top and bottom) of ChIs in the striatum of ChAT$^{Cre/+}$ animals transfected with AAV5-ChR2. ChR2-eYFP (green, left) shows colocalisation with ChAT (red, middle), see merge (right).

3. Initial release probability and nAChRs interact to control the short-term plasticity of striatal DA release

is manipulated, for example by changing extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) (Manabe *et al.* 1993; Thomson *et al.* 1993; Thomson 1997), or across synapses with varying P_r (Thomson *et al.* 1993; Dobrunz and Stevens 1997; Ouardouz and Lacaille 1997). DA release *in vitro* shows strong short-term depression (Kennedy *et al.* 1992; Cragg 2003), but the nature of this depression is not well-characterised. In this chapter I have explored the extent to which P_r controls the short-term plasticity of DA release.

3.1.2 Network factors

In addition to presynaptic terminal factors, such as P_r, transmitter release and the short-term plasticity of release can also be controlled by local circuitry via receptors expressed on presynaptic terminals. DA release and the short-term plasticity of release are known to be strongly modulated by ACh, released by striatal ChIs and acting at nAChRs on DA terminals. ACh acting at nAChRs can directly drive DA release, even in the absence of DA neuron firing activity (Threlfell *et al.* 2012). nAChRs also strongly modulate the short-term plasticity of DA release (Rice and Cragg 2004; Zhang and Sulzer 2004), as described in chapter 1.2.3. Briefly, when nAChRs are active, initial DA release is high, but subsequent release over the following tens to hundreds of milliseconds is very low. When nAChRs are inhibited, removing the confounding effects of ACh at the DA terminal, depression is relieved and DA release shows facilitation over tens of milliseconds which decays over a timescale of 50-100 ms (see **Fig. 1.4**). This has been hypothesised to be due to the effect of nAChRs on P_r (Zhang and Sulzer 2004; Threlfell *et al.* 2012). It is proposed that nAChR activity increases P_r ,so vesicles are rapidly depleted and release shows strong depression; conversely when nAChRs are inhibited, P_r is decreased, reducing vesicle depletion and relieving short-term depression. To date however, the effect of P_r on DA release and the short-term plasticity of DA release has mostly been explored without taking into account the effects of ACh, and the mechanism underlying the effect of nAChRs on the short-term plasticity of DA release has not been explored (Cragg 2003; Montague *et al.* 2004; Zhang *et al.*

2009). Given the behaviourally significant coincident changes in ChI and DA neuron activity *in vivo* (Morris *et al.* 2004), discussed in chapter 1.1.4.2, it is important to understand the control of DA release and the short-term plasticity of release both when nAChRs are active and inactive. Therefore, here I have explored whether there is a relationship between P_r and the short-term plasticity of DA release, and whether nAChRs control the short-term plasticity of DA release via their effects on P_r.

3.1.3 Striatal regions

As discussed in chapter 1.1.3, the striatum receives topographic dopaminergic inputs from the midbrain, with inputs from the SNc largely innervating the dorsal and lateral striatum and inputs from the VTA largely innervating the ventral striatum and NAc (Haber 2003). These different populations of DA neurons express different complements of ion channels and other proteins, which may influence DA release (Liss and Roeper 2008). Particularly relevantly here, P_r is thought to vary with striatal region (Cragg 2003; Zhang *et al.* 2009). Therefore, in this chapter, the short-term plasticity of DA release has been explored in both dlCPu and NAcC.

3.1.4 Summary

This chapter explores how P_r interacts with the actions of ACh at nAChRs to control the short-term plasticity of DA release, in different striatal regions.

3.2 Methods

Methods were carried out as described in chapter 2, unless otherwise described here.

3.2.1 Animals

As described in chapter 2.1, the majority of experiments in this chapter have used adult male C57Bl6/J mice. Some experiments have used an optogenetic approach using adult male $DAT^{Cre/+}$

mice transfected with AAV5-ChR2, as described in chapter 2.3.2.4. All procedures were carried out in accordance with UK Home Office project licence and local guidelines.

3.2.2 Slice preparation and FCV

Acute striatal slices were prepared and FCV carried out as described in chapter 2. In some experiments the composition of aCSF was modified. Control aCSF contained 5 mM K^+ and 2.4 mM Ca^{2+}, containing, in mM: 124.3 NaCl, 3.8 KCl, 1.2 KH_2PO_4 and 2.4 $CaCl_2$. Where $[Ca^{2+}]_o$ was varied, $[CaCl_2]$ was varied appropriately. Where extracellular potassium concentration ($[K^+]_o$) was varied, the following components differed from control: 1.25 mM $[K^+]_o$ aCSF contained, in mM: 128.0 NaCl and 0.05 mM KCl; 7.5 mM $[K^+]_o$ aCSF contained, in mM: 121.8 NaCl and 6.3 mM KCl.

3.2.3 Stimulation and experimental design

DA release was evoked using either using electrical or light stimulation, as described in chapter 2.3.

To investigate the short-term plasticity of DA release, the majority of experiments in this chapter have applied single (1p) or paired pulse stimulations across a range of interpulse intervals (IPIs) (2p, 10-200 ms) in a single recording site per slice in pseudorandomized order and in triplicate. Peak $[DA]_o$ evoked by 1p stimulation has been used as a measure of P_r (Cragg 2003). The paired-pulse ratio (P2/P1) has been used as a measure of the short-term plasticity of DA release. Since $[DA]_o$ evoked by a 2nd pulse, P2, with short IPIs (less than approximately 1 s), summates with $[DA]_o$ released by the 1st pulse, P2 was determined by subtracting $[DA]_o$ evoked by a single pulse from $[DA]_o$ evoked by two pulses (P1+P2) (Cragg 2003) (**Fig. 3.1**). P2/P1 could then be determined.

In optogenetic experiments, alternating single or paired-pulse (40 ms IPI) LED stimulations were applied, and P2/P1 determined as described above.

In experiments where aCSF composition was varied, recordings were first carried out in control aCSF, followed by the lowest ion concentration aCSF, then with increasing ion concentrations, including a repeat of control aCSF. Slices were equilibrated in each ion concentration for at least 20 mins before data was included in analysis, by when 1p $[DA]_o$ was reproducible.

In experiments where stimulation current was varied, stimuli were applied either at a perimaximal current (I_{100}, 0.6 mA), as described in chapter2.3, or at a lower current (I_{50}, 0.015-0.3 mA), where peak $[DA]_o$ evoked by 1p was approximately half (40-60%) that evoked by a 1p I_{100} stimulation.

The above experiments were all carried out in a single recording site in either dlCPu or NAcC. To explore relationships between P_r and P2/P1 across recording sites, data from all these recording sites (1 per animal) were pooled, and the data analysed.

3.2.4 Data analysis

Data were acquired and analysed as described in chapter 2.2.2. Comparisons for means were carried out in GraphPad Prism using One- and Two-way ANOVAs and *post hoc* Bonferroni's t-tests. Correlation analysis was carried out using linear regression analysis in GraphPad Prism. *n* = number of animals.

3.2.5 Drugs

Dihydro-β-erythroidine (DHβE) was purchased from Tocris Biosciences or Ascent Scientific and dissolved in distilled water to make stock aliquots 1000x final concentrations and stored at -20^{o}C. The final concentration was made up in aCSF immediately before use.

3.3 Results

In this chapter I have explored the relationship between P_r and the short-term plasticity of DA release, with and without nAChR inhibition, in dlCPu and NAcC.

3.3.1 nAChRs control DA release plasticity

Firstly, the control of the short-term plasticity of DA release by nAChRs, which has been previously reported (Rice and Cragg 2004; Zhang and Sulzer 2004), was confirmed. Without nAChR inhibition, DA release showed strong depression (P2/P1 < 0.5) across all IPIs, in both regions (**Fig. 3.2**). This depression was strongest at the shortest IPIs, with P2/P1 increasing slightly with increasing IPI (**Fig. 3.2**). nAChR inhibition, using the β2* nAChR antagonist DHβE, decreased $[DA]_o$ evoked by 1p (**Fig. 3.2 a,b**), and relieved paired-pulse depression (**Fig. 3.2 c,d**)(Rice and Cragg 2004). nAChR inhibition significantly increased P2/P1 at 10-40 ms IPI in dlCPu and at all IPIs in NAcC (Two-way ANOVA, *post hoc* Bonferroni t test, *p<0.001*; *n = 3*). nAChR inhibition revealed an inverse relationship between P2/P1 and IPI, with DA release showing facilitation at short IPIs (10 ms in dlCPu (**Fig. 3.2 c**), 10-25 ms in NAcC (**Fig. 3.2 d**)), which decayed to depression at longer IPIs. This relationship was slightly different between the two striatal regions. P2/P1 was higher in NAcC than dlCPu at 25-100 ms IPI (Two-way ANOVA, *post hoc* Bonferroni t test, *p<0.001*; *n = 3*) (**Fig. 3.2 e**), suggesting that facilitation may decay slightly slower, or short-term depression may be slightly weaker in NAcC vs. dlCPu.

3.3.2 When nAChRs are active, there is no relationship between P_r and the short-term plasticity of release

3.3.2.1 Modulating $[Ca^{2+}]_o$

The relationship between P_r and the short-term plasticity of release was first explored in drug-free conditions, that is with nAChRs active. Firstly, P_r was varied by manipulating $[Ca^{2+}]_o$, and the effect on the short-term plasticity of DA release was explored. As described in chapter 1.2.1,

synchronous transmitter release is driven by Ca^{2+} which enters the presynaptic terminal via VGCCs. Varying $[Ca^{2+}]_o$ alters the driving force for Ca^{2+} entry, altering Ca^{2+} influx and hence the probability of transmitter release. Therefore, as expected, and as described previously (Bull *et al.* 1990; Chen and Rice 2001; Cragg 2003; Ford *et al.* 2010), $[DA]_o$ evoked by all stimulations was strongly dependent on $[Ca^{2+}]_o$ in both striatal regions (**Fig. 3.3 a,b**). Halving $[Ca^{2+}]_o$ from 2.4 to 1.2 mM, decreased $[DA]_o$ evoked by 1p by approximately 80% in dlCPu and 60% in NAcC. On the other hand, increasing $[Ca^{2+}]_o$ from 2.4 to 3.6 mM increased $[DA]_o$ evoked by 1p by approximately 100% in dlCPu and 35% in NAcC. Despite these large changes in P_r, changing $[Ca^{2+}]_o$ had relatively small effects on P2/P1 (**Fig. 3.3 c,d**). In dlCPu, there was a significant interaction between $[Ca^{2+}]_o$ and IPI (Two-way ANOVA, $p < 0.05$; $n = 3$), but no significant effect of $[Ca^{2+}]_o$ at any given IPI (*post hoc* Bonferroni t test, $p > 0.05$; $n = 3$) (**Fig. 3.3 c**). In NAcC, decreasing $[Ca^{2+}]_o$ from 2.4 to 1.2 mM increased P2/P1 at 10 and 40 ms IPI (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.05$; $n = 3$), but increasing $[Ca^{2+}]_o$ had no significant effect at any IPI (Two-way ANOVA, *post hoc* Bonferroni t test, $p > 0.05$; $n = 3$) (**Fig. 3.3 d**). Despite large variations in $[DA]_o$ evoked by 1p, DA release showed strong paired-pulse depression in all $[Ca^{2+}]_o$, with P2/P1 below 0.25 in dlCPu and below 0.5 in NAcC at all IPIs.

However, Ca^{2+} plays many roles presynaptically in addition to controlling P_r. For example $[Ca^{2+}]$ controls vesicle trafficking and endocytosis (Schweizer and Ryan 2006) and facilitation of release (Katz and Miledi 1967; Balnave and Gage 1974; Thomson 2000). Thus, varying $[Ca^{2+}]_o$ may influence these factors in addition to altering P_r. Hence the effect of modulating P_r using other methods was also explored.

3.3.2.2 Modulating stimulation current

P_r was modulated by varying stimulation current in drug-free conditions and the effect on P2/P1 explored. Stimulations were applied, either at a perimaximal current (I_{100}) or at a current that approximately halved $[DA]_o$ evoked by 1p at a perimaximal current (I_{50}) (**Fig. 3.4 a,b**). Again,

despite large effects on peak $[DA]_o$ evoked by 1p, this manipulation had little effect on the short-term plasticity of DA release. There was no significant effect of stimulation current on P2/P1 in either region (Two-way ANOVA, $p > 0.05$; $n = 3$) (**Fig. 3.4 c,d**). At both stimulation currents, DA release showed strong paired-pulse depression at all IPIs, with P2/P1 below 0.25 in dlCPu and below 0.5 in NAcC.

3.3.2.3 Modulating $[K^+]_o$

P_r was then modulated by varying $[K^+]_o$ in drug-free conditions, and the effect on P2/P1 explored. Surprisingly, since increased $[K^+]_o$ usually results in depolarisation of neurons and hence increased excitability and transmitter release (Gage and Quastel 1965; Cooke and Quastel 1973), decreasing $[K^+]_o$ from 5 to 1.25 mM increased peak $[DA]_o$ evoked by all stimulations in both regions. Conversely, increasing $[K^+]_o$ from 5 to 7.5 mM, decreased $[DA]_o$ (**Fig 3.5 a,b**). However, varying $[K^+]_o$ had little effect on the short-term plasticity of release. In dlCPu, there was no effect of $[K^+]_o$ on P2/P1 (Two-way ANOVA, $p > 0.05$; $n = 3$) (**Fig. 3.5 c**). In NAcC, there was a significant interaction between $[K^+]_o$ and IPI (Two-way ANOVA, $p < 0.01$; $n = 3$), but no significant effect at any given IPI (*post hoc* Bonferroni t-test, $p > 0.05$) (**Fig. 3.5 d**). In all $[K^+]_o$ though, in both regions, DA release still showed strong short-term depression of release, with P2/P1 below 0.25 in dlCPu and below 0.5 in NAcC at all IPIs.

3.3.2.4 Across release sites

At many synapse types, variations in P_r contribute to variations in the short-term plasticity of release between synapses (Debanne *et al.* 1996; Dobrunz and Stevens 1997). Short-term plasticity of release is known to be highly heterogeneous between striatal DA release sites (Cragg 2003; Daniel *et al.* 2009). Therefore, it was investigated whether P_r might contribute to this heterogeneity by examining the correlation between P_r and P2/P1 across all sampled release sites, in drug-free conditions. In both regions, P2/P1 increased with increasing IPI in both regions (**Fig. 3.6 a,b**), as seen in **Fig. 3.2 c,d**. However, there was no significant correlation between

P2/P1 and $[DA]_o$ evoked by 1p at any IPI in either striatal region (linear regression analysis, $p > 0.05$, $0 < R^2 < 0.04$; $n = 25\text{-}35$) (**Fig. 3.6 a,b**).

Therefore, when nAChRs are active, DA release appears to be clamped, such that strong paired-pulse depression is always exhibited, and there is no relationship between initial DA release probability and the short-term plasticity of DA release, either at a given release site or across release sites.

3.3.3 When nAChRs are inhibited, there is a slight relationship between P_r and the short-term plasticity of release at short IPIs:

As discussed above, nAChRs are strong determinants of DA release, but it is not known how they interact with other factors to control DA release plasticity. Hence the relationship between P_r and short-term plasticity of release was next explored when the confounding effects of nAChRs were inhibited. All the following experiments were carried out in the presence of the nAChR antagonist, DHβE.

3.3.3.1 Modulating $[Ca^{2+}]_o$

P_r was first modulated by varying $[Ca^{2+}]_o$. As expected, $[DA]_o$ showed a strong $[Ca^{2+}]_o$ dependence across all stimulations in both regions (**Fig. 3.7 a,b**). Halving $[Ca^{2+}]_o$ from 2.4 to 1.2 mM, decreased peak $[DA]_o$ evoked by 1p by approximately 65% in dlCPu and 45% in NAcC. Conversely, increasing $[Ca^{2+}]_o$ from 2.4 to 3.6 mM increased peak $[DA]_o$ evoked by 1p by approximately 80% in dlCPu and 40% in NAcC. Changing $[Ca^{2+}]_o$ had relatively subtle effects on the short-term plasticity of release. In dlCPu, there was no significant effect of $[Ca^{2+}]_o$ on P2/P1 (Two-way ANOVA, $p > 0.05$; $n = 4$) (**Fig. 3.7 c**). In NAcC, P2/P1 was significantly increased in 1.2 vs. 2.4 mM $[Ca^{2+}]_o$ and decreased in 3.6 vs. 2.4 mM $[Ca^{2+}]_o$ at 10 ms IPI (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.05$; $n = 3$) (**Fig. 3.7 d**). There was no effect of $[Ca^{2+}]_o$ at longer IPIs (Two-way ANOVA, *post hoc* Bonferroni t test, $p > 0.05$; $n = 3$). Therefore, when P_r was modulated by varying $[Ca^{2+}]_o$, there was an inverse relationship between P_r and P2/P1 at the shortest IPIs, in

NAcC. This can be seen more clearly by plotting P2/P1 at 10 ms IPI only, against peak $[DA]_o$ evoked by 1p (**Fig. 3.7 e,f**). With nAChR inhibition there appeared to be an inverse relationship between P2/P1 at 10 ms IPI, and peak $[DA]_o$ evoked by 1p, in both regions, which appears slightly stronger in NAcC. This was in contrast to drug-free conditions (data from **Fig. 3.3**), where there was very little relationship between P2/P1 at 10 ms IPI and $[DA]_o$ evoked by 1p in either region (**Fig. 3.7 e,f**). Together, these data suggest that the extent to which other manipulations of P_r control the short-term plasticity of DA release depends on the activity of nAChRs.

Additionally, these data suggest that the control of the short-term plasticity of DA release by nAChRs is not simply dependent on their effect on P_r. Despite comparable 1p $[DA]_o$ in high $[Ca^{2+}]_o$ with nAChR inhibition, and in low $[Ca^{2+}]_o$ without nAChR inhibition, P2/P1 is markedly different between these two conditions: DA release exhibits facilitation when nAChRs are inhibited and strong depression when they are not (**Fig. 3.7 e,f**). This suggests that nAChRs do not simply control the short-term plasticity of DA release by increasing P_r and hence decreasing subsequent release.

It is also interesting to note that modulating $[Ca^{2+}]_o$ appeared to have greater effects on $[DA]_o$ evoked by 1p in dlCPu than NAcC (**Fig. 3.7 a,b**), yet P2/P1 appeared to be modulated more strongly in NAcC than dlCPu (**Fig. 3.7 e,f**). This further suggests that the short-term plasticity of DA release can be controlled independently of P_r.

3.3.3.2 Modulating stimulation current

P_r was also varied by changing stimulation current, when nAChRs were inhibited. Single or paired-pulse stimulations were applied at I_{100} and I_{50} (**Fig. 3.8 a,b**). Decreasing stimulation current significantly increased P2/P1 at 10 ms IPI in dlCPu and at 10-40 ms IPI in NAcC (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.01$; $n = 3$) (**Fig. 3.8 c,d**). Therefore again, when nAChRs are inhibited, there appears to be an inverse relationship between P_r and P2/P1 at very short IPIs.

3.3.3.3 Across release sites

The relationship between P_r and the short-term plasticity of DA release was then examined across all recording sites in the presence of DHβE, to further investigate the relationship between these two parameters and explore if differences in P_r may contribute to heterogeneity in P2/P1 between recording sites (Cragg 2003), when nAChRs are inhibited. There was a significant inverse correlation between P2/P1 and peak $[DA]_o$ evoked by 1p at 10 ms IPI in dlCPu (linear regression, $p < 0.001$, slope = -0.79, $R^2 = 0.48$; $n = 35$) (**Fig. 3.9 a**) , and at 10 and 40 ms IPI in NAcC, although this correlation was strongest at 10 ms IPI (linear regression, $p < 0.05$, 10 ms: slope = -0.52, $R^2 = 0.30$; $n = 25$; 40 ms: slope = -0.19, $R^2 = 0.21$; $n = 25$) (**Fig. 3.9 b**). There was no significant relationship between P2/P1 and $[DA]_o$ evoked by 1p at longer IPIs in either region (linear regression, $p > 0.05$, $R^2 < 0.1$; $n = 25\text{-}35$) (**Fig. 3.9 a,b**).

Therefore, DA transmission does show the classic inverse relationship between initial release, or P_r, and subsequent release reported in other transmitter systems (Thomson et al., 1993, Thomson, 1997), but only when nAChRs are not active and only at the shortest IPIs. At longer IPIs, longer than 25-40 ms, there was no significant relationship between these parameters, suggesting that the short-term depression of DA release is release-independent over this time course.

3.3.4 When nAChRs are inhibited, P_r and the short-term plasticity of release are dissociable:

3.3.4.1 Modulating $[K^+]_o$

When nAChRs were inhibited, modulating $[K^+]_o$ had no significant effect on peak $[DA]_o$ evoked by 1p in either region (One-way ANOVA, $p > 0.05$; $n = 3$) (**Fig. 3.10 a,b**). However, in dlCPu, P2/P1 was significantly increased at 25 and 40 ms IPI in 1.25 vs. 5 mM $[K^+]_o$ and decreased at all IPIs in 7.5 vs. 5 mM $[K^+]_o$ (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.001$; $n = 3$) (**Fig. 3.10 c**). In NAcC, there was no significant interaction between $[K^+]_o$ and IPI (Two-way ANOVA, $p > 0.05$; $n =$

3) but there was a significant main effect of $[K^+]_o$ on P2/P1 (Two-way ANOVA, $p < 0.001$; $n = 3$), with P2/P1 increased at most IPIs in 1.25 vs. 5 mM $[K^+]_o$ and decreased at all IPIs in 7.5 vs. 5 mM $[K^+]_o$ (**Fig. 3.10 d**). Hence, with the manipulation of $[K^+]_o$, there was no clear relationship between P2/P1 at short IPIs and $[DA]_o$ evoked by 1p, either with or without nAChR inhibition, in either region (**Fig 3.10 e,f**). Manipulating $[K^+]_o$ is therefore able to independently control P_r and the short-term plasticity of DA release, demonstrating that even when nAChRs are inhibited, these two parameters are dissociable.

3.3.5 In the absence of network factors, P_r does not strongly control the short-term plasticity of DA release in either striatal region

DA release is known to be differentially controlled by a number of presynaptic and network factors in dlCPu vs. NAcC (Cragg 2003; Zhang *et al.* 2009; Anwar *et al.* 2011). Several of the above results hinted that the relationship between P_r and the short-term plasticity of release, seen when nAChRs are inhibited, might be stronger, and extend to longer IPIs, in NAcC than in dlCPu. For example, this relationship appeared stronger when examined by manipulating $[Ca^{2+}]_o$ (**Fig. 3.7 c,d**), and is apparent at longer IPIs when examined either by manipulating stimulation current (**Fig. 3.8 c,d**) or across release sites (**Fig. 3.9**). Therefore, I wanted to explore directly whether there was a difference in the relationship between P_r and the short-term plasticity of release between striatal regions, in the absence of all network factors. The above experiments have used the nAChR antagonist DHβE to block the confounding effects of ACh actions, but glutamate and GABA transmission are not inhibited. Therefore, an optogenetic approach was used to completely remove any confounding circuit effects, and allow the direct investigation of the short-term plasticity of release dependent on presynaptic factors within DA terminals. ChR2 was selectively expressed in DA neurons by transfecting the midbrain of $DAT^{Cre/+}$ mice with AAV5-ChR2, as described in chapter 2.3.2. Single pulse or paired pulse (40 ms IPI) light stimulations were applied in different $[Ca^{2+}]_o$ (0.5-3.6 mM). As discussed in chapter 2.3.2, shorter IPIs cannot be used in optogenetic experiments due to the intrinsic kinetics of ChR2. However, this slightly

longer IPI of 40 ms is more representative of IPIs reported during DA neuron burst firing *in vivo* (Grace and Bunney 1984), and hence may be more physiologically relevant. As expected, increasing $[Ca^{2+}]_o$ increased peak $[DA]_o$ evoked by both single and paired pulse stimulations, in both regions (**Fig. 3.11 a,b**). However, there was no relationship between P2/P1 (40 ms IPI) and either $[Ca^{2+}]_o$ (**Fig. 3.11 c**), or peak $[DA]_o$ evoked by 1p (**Fig. 3.11 d**) in either region. This suggests that at this longer, and perhaps more physiologically relevant IPI than the shortest IPIs used previously, and in the absence of any confounding network effects, P_r is not a strong determinant of the short-term plasticity of DA release, either in dlCPu or NAcC.

3.4 Discussion

It is necessary to understand the control of the short-term plasticity of DA release in order to understand how behaviourally significant firing patterns in DA neurons are translated in striatal DA release. In this chapter I have explored how P_r interacts with nAChRs in the control of the short-term plasticity of striatal DA release.

3.4.1 When nAChRs are active, P_r has little effect on the short-term plasticity of release

As reported previously, when nAChRs were active, DA release showed very strong short-term depression across all IPIs (10-200 ms) (Rice and Cragg 2004; Zhang and Sulzer 2004). In these conditions, there was little relationship between P_r and the short-term plasticity of DA release at any IPI. This was firstly seen by manipulating P_r at a given release site. Manipulating $[Ca^{2+}]_o$, stimulation current, or $[K^+]_o$ greatly altered peak $[DA]_o$ evoked by 1p but had very little effect on the short-term plasticity of release. In NAcC, decreasing P_r by decreasing $[Ca^{2+}]_o$ did increase P2/P1 at very short IPIs, suggesting there may be a very weak inverse relationship between P2/P1 and P_r at this time point. There was no effect on P2/P1 of increasing P_r by increasing $[Ca^{2+}]_o$, but this may be due to a floor effect, since P2/P1 was already incredibly low. However, no

other manipulation significantly modulated P2/P1, at any IPI, in either region. When nAChRs were active, release always showed strong short-term depression at all IPIs, with P2/P1 below 0.25 in dlCPu and below 0.5 in NAcC.

Secondly, the lack of relationship between P_r and the short-term plasticity of release when nAChRs were active was seen by looking across release sites. $[DA]_o$ is highly heterogeneous across striatal recording sites, likely due to differential innervation density and heterogeneous P_r between striatal terminals (Cragg 2003; Daniel *et al.* 2009). However, despite relatively large variations in $[DA]_o$ across recording sites, suggestive of large variations in P_r, there was no correlation between P2/P1 and peak $[DA]_o$ evoked by 1p, at any IPI investigated, in either region.

Together these results suggest that, when nAChRs are active, P_r is not a strong determinant of DA release plasticity. Instead, when nAChRs are active, DA release shows strong short-term depression across the 10-200 ms IPI time course, which is not strongly modulated by P_r, and is therefore a release-independent form of depression.

3.4.2 When nAChRs are inhibited, P_r weakly controls short-term plasticity of release

As reported previously, inhibiting nAChRs relieved short-term depression (Rice and Cragg 2004; Zhang and Sulzer 2004; Zhang *et al.* 2009), such that there was an inverse relationship between IPI and P2/P1, with DA release showing facilitation of release over very short IPIs which decayed to depression over the 10-200 ms time course. With nAChRs inhibited, there was an inverse relationship between P_r and P2/P1 at very short IPIs. Firstly, at a given release site, changing $[Ca^{2+}]_o$ or stimulation current greatly altered peak $[DA]_o$ evoked by 1p, and inversely modulated P2/P1 at the shortest IPIs. Secondly, looking across release sites, there was a significant inverse correlation between P2/P1 and peak $[DA]_o$ evoked by 1p at 10 ms IPI in dlCPu and 10-40 ms IPI in NAcC. Therefore, P_r does correlate with the short-term plasticity of DA release, suggesting that

DA release does exhibit a release-dependent component of plasticity, but only when nAChRs are not active, and over very short timescales.

At longer IPIs, when nAChRs were inhibited, DA release showed paired-pulse depression, which was not modulated by P_r. Therefore at longer IPIs, a release-independent form of depression dominates control of DA release, even when nAChRs are inhibited.

Even at short IPIs and when nAChRs are inhibited, the extent to which P_r controls the short-term plasticity of DA release appears relatively weak. In certain conditions P_r and P2/P1 were found to be dissociable; when nAChRs were inhibited, manipulating $[K^+]_o$ had very little effect on $[DA]_o$ evoked by 1p, yet significantly altered P2/P1 across IPIs, particularly in dlCPu. Additionally, although manipulating $[Ca^{2+}]_o$ or stimulation current demonstrated a relationship between P_r and P2/P1 at short IPIs, these manipulations had large effects on $[DA]_o$ evoked by 1p, but relatively small effects on P2/P1, with DA release showing facilitation at short IPIs in all conditions. This is in contrast to the strong effects of nAChR actions on P2/P1. nAChR inhibition has comparable effects on peak $[DA]_o$ evoked by 1p to modulations of P_r but much greater effects on P2/P1. When nAChRs are active, DA release shows very strong short-term depression, but at short IPIs nAChR inhibition switches this to facilitation. This contrast between the effects of P_r and nAChRs on the short-term plasticity of release can be visualised in **Fig. 3.7 e,f**. This figure shows that there is not a continuous trend between data obtained with and without nAChR inhibition, but instead they form two distinct data sets. These data suggest that nAChRs control the short-term plasticity of DA release much more strongly than P_r does. Additionally, these data therefore suggest that nAChRs are not simply controlling the short-term plasticity of DA release by altering P_r, as has been previously thought (Zhang and Sulzer 2004; Threlfell *et al.* 2010). Instead, nAChRs appear to clamp subsequent DA release such that it always exhibits strong short-term depression and is invariant with other manipulations of P_r.

3.4.3 The short-term plasticity of DA release and possible underlying mechanisms

This chapter has shown that DA release is highly dynamic and exhibits at least four components of short-term plasticity over the timescale of 10-200 ms. When nAChRs are not active DA release exhibits release-independent depression at IPIs > 40 ms, facilitation at very short IPIs, and a release-dependent component at very short IPIs. When nAChRs are active, DA release exhibits release-independent depression at all IPIs. Possible underlying mechanisms for these components are now discussed.

Firstly, both when nAChRs are active and inactive, DA release shows strong depression over the timescale of 40-200 ms, which is not modulated by P_r and hence is a form of release-independent depression. This depression has previously been reported to recover over a timescale of many seconds (Abeliovich *et al.* 2000). This depression has been suggested to be due to vesicle depletion, with its slow recovery corresponding to the time required for recapturing and refilling of vesicles (Abeliovich *et al.* 2000). However, since this component of plasticity shows no correlation with levels of initial release, this mechanism seems unlikely. Another possible mechanism is the activation of metabotropic receptors located on DA terminals. As described previously, activation of D_2Rs decreases subsequent DA release and contributes to depression of release (Schmitz *et al.* 2002; Bello *et al.* 2011). However, the reported timecourse of D_2R effects (250 ms-2 s) (Benoit-Marand *et al.* 2001; Phillips *et al.* 2002; Schmitz *et al.* 2002) is too slow to underlie the depression reported here at 40-200 ms. Additionally, D_2R antagonists reduce, but do not abolish, depression of DA release (Mayer *et al.* 1988; Kennedy *et al.* 1992; Cragg 2003) see **Fig. 4.10, 4.11**). It is possible that other metabotropic receptors, such as $GABA_B$ receptors or mGluRs may contribute to this depression. Again though, this seems unlikely, as it has been reported that glutamate and GABA transmission do not affect DA release evoked by the short stimulations as used here (Avshalumov *et al.* 2003). Additionally, in this chapter, depression of release was observed even when an optogenetic approach was used to selectively activate DA

neurons, removing all network effects on DA release. One mechanism thought to underlie release-independent depression at other central synapses is an activity-dependent change in ion channel conductances (Thomson 2000; Catterall and Few 2008). For example, some VGCCs show Ca^{2+}-dependent inactivation, leading to a decrease in Ca^{2+} entry, and hence transmitter release, with subsequent action potentials (Dobrunz *et al.* 1997; Forsythe *et al.* 1998; Thomson and Bannister 1999; Xu and Wu 2005). Alternatively, certain K^+ channels are activated by Ca^{2+}, leading to a decrease in the preterminal excitability or the amplitude of subsequent action potentials, and hence reduced subsequent release (Dodson and Forsythe 2004). Indeed, action potentials in DA neuron bursts are known to progressively decrease in amplitude (Grace and Bunney 1984), which may reduce the time available for Ca^{2+} entry, and hence decrease subsequent DA release. In this chapter, the only manipulation that modulated the short-term plasticity of release at IPIs longer than 40 ms was changing $[K^+]_o$, which may support a role for changes in K^+ conductance in this depression. However, changes in ion conductance are typically short-lived, affecting release at timescales of up to several hundred milliseconds (Royer *et al.* 2000; Thomson 2000; Catterall and Few 2008), which would seem incompatible with the slow recovery of DA release from depression, although some small changes in membrane excitability and transmitter release have been reported to persist for several seconds (Brody and Yue, 2000, Xu and Wu, 2005). An alternative mechanism that has been proposed to underlie release-independent depression at a non-vertebrate synapse is the 'switching off' of a population of synapses following stimulation (Royer *et al.* 2000). It is possible that such a refractory mechanism, where only certain populations of active zones are available for release, may operate at DA synapses, with active zones gradually 'turned on' again over the subsequent 30 s or so.

Secondly, when nAChRs are not active, DA release shows short-term facilitation which decays over approximately 10-40 ms. One possible mechanism is residual Ca^{2+} from the first action potential summating with Ca^{2+} entry driven by a subsequent action potential to enhance Ca^{2+}-dependent release. This is the thought to be the most common mechanism underlying

facilitation at glutamatergic synapses (Thomson 2000; Zucker and Regehr 2002). Other possible mechanisms include the Ca^{2+}-dependent facilitation of VGCCs (Catterall and Few 2008), or the activity-dependent broadening of action potentials, which is known to occur in DA neurons (Grace and Bunney 1984), which may prolong the period of Ca^{2+} entry into the terminal. The time course of facilitation reported here is slightly more rapid than that reported for glutamatergic synapses, which usually decays over 50-100 ms (Thomson 1997). This may reflect differences in protein expression between these types of synapses, for example in proteins which control Ca^{2+} buffering or extrusion. Alternatively, the processes underlying facilitation may decay with a similar timecourse in DA and glutamatergic synapses, but are masked by the strong depression of DA release at longer IPIs.

Next, when nAChRs are not active, DA release shows a release-dependent component at very short IPIs, demonstrated by the classic inverse relationship between P_r and P2/P1 (Thomson 2000). As at other central synapses, this is most likely due to depletion of readily releasable, docked vesicles. The more of these vesicles that are released by an initial action potential, the fewer remain, and hence the lower subsequent release. The fact that this component of plasticity is only apparent at very short IPIs may suggest that DA release sites are only briefly refractory, or can be rapidly replenished by a pool of recycling vesicles. Alternatively, it may reflect the dominance of the mechanism underlying strong, release-independent depression at longer IPIs.

Finally, DA release shows a very strong nAChR-dependent depression. It has been previously hypothesised that this nAChR-dependent depression is due to the effects of nAChRs on P_r (Zhang and Sulzer 2004; Threlfell *et al.* 2012). However,the data presented in this chapter challenge this hypothesis. The data presented here suggest that ACh, via nAChRs, does not simply modulate the short-term plasticity of DA release by increasing P_r, and hence decreasing the number of vesicles available for subsequent release, since nAChR inhibition has very different effects on the short-

term plasticity of DA release compared to other manipulations of P_r. Instead, nAChRs appear to clamp release, inhibiting facilitation and preventing subsequent DA release. The mechanism underlying this is not certain. nAChRs are relatively un-selective cation channels, permeable to Na^+, K^+ and, to a limited extent, Ca^{2+}. Therefore, when active they will allow increased Na^+ and Ca^{2+} entry into the presynaptic zone. However, Na^+ and Ca^{2+} entry into the presynaptic terminal following an action potential will always be high, so it is not obvious how increasing the entry of these ions could so forcefully clamp release. Many synaptic ion channels and receptors form specific protein-protein interactions, including with Ca^{2+}-sensitive presynaptic proteins that modulate vesicle fusion, such as syntaxin-1 or Munc proteins (Binda *et al.* 2005; Chan *et al.* 2007; Khanna *et al.* 2007; Catterall 2010). Little is known about the protein-protein interactions of nAChRs. It is conceivable that local Ca^{2+} entry via nAChRs could interact with Ca^{2+}-sensitive proteins held near nAChRs by protein-protein interactions, which subsequently act to clamp transmitter release. A more likely hypothesis may be that nAChRs greatly increase the conductance of the axon and presynaptic zone, such that the membrane resistance and hence the excitability of the axon or terminal is decreased, and subsequent action potentials fail to reach the terminal. This would explain the almost complete lack of subsequent DA release following an initial stimulus when nAChRs are active, if subsequent stimuli fail to reach the presynaptic zone. Action potential conduction failure has been proposed to underlie release-independent depression at several glutamatergic synapses (Brody and Yue 2000; He *et al.* 2002; Muñoz-Cuevas *et al.* 2004) and may play a role in DA axons too when nAChRs are active. Given the impracticalities of electrophysiological axonal recordings it will be difficult to test whether action potential conduction failure does indeed underlie the effects of nAChRs on DA release. Possible approaches to explore this may include using genetically encoded voltage-sensitive dyes or extracellular recordings of striatal activity before and after nAChR inhibition.

3.4.4 Short-term plasticity of DA release in different striatal regions

Throughout this chapter I have investigated DA release in both dlCPu and NAcC. The short-term plasticity of DA release is grossly similar in these two regions, displaying the same four components of plasticity. However, the relative importance and time course of each component appears to vary slightly; in particular, facilitation appeared to decay more slowly in NAcC than in dlCPu, and the release-dependent component of plasticity, apparent when nAChRs were inhibited, seemed to be stronger and visible at longer IPIs in NAcC than dlCPu. Therefore, an optogenetic approach was used to explore directly whether at the slightly longer, and possibly more physiologically relevant, IPI of 40 ms, in the absence of all confounding network factors, there was a stronger relationship between P_r and the short-term plasticity of DA release in NAcC vs. dlCPu. However, this approach demonstrated no significant relationship between P_r and the short-term plasticity of DA release at 40 ms IPI in either striatal region. Differences between this experiment and electrical experiments, which showed a significant inverse relationship at 40 ms IPI in NAcC, may suggest that other network effects, such as glutamate or GABA transmission, which are present in electrical but not optogenetic experiments, contribute to the control of the short-term plasticity of DA release. Alternatively it may be due to slight differences in the animals used. $DAT^{Cre/+}$ animals used in optogenetic experiments have slightly decreased DAT expression (Backman 2006), which may lead to compensatory changes in DA release. However, even in electrical experiments the relationship between P_r and P2/P1 at 40 ms IPI is very slight even in NAcC (**Fig. 3.7, 3.8, 3.9**). Therefore, overall, at physiologically representative IPIs, P_r appears to play little role in controlling the short-term plasticity of DA release in either striatal region.

3.4.5 Implications

As discussed previously, DA neurons switch from low frequency to bursts of high frequency firing (15-25 Hz or higher) in response to behaviourally salient or reward-related events (Schultz 2007). The short-term plasticity of DA release will be key in determining how these changes in firing

pattern translate into differences in striatal DA release. When DA release shows strong short-term depression at relatively short IPIs, a burst of DA neuron activity will not release much more DA than a single action potential or low frequency firing. Conversely, if DA release shows facilitation at relatively short IPIs which decays with increasing IPI, a switch from tonic to burst firing will lead to large increases in striatal DA, and hence the contrast between burst- and tonic-evoked DA signals will be large. This will impact on the target neurons of DA signals: a greater contrast between burst- and low frequency-evoked DA signals will be more easily detected by MSNs, such that the switch in DA neuron firing pattern will have more impact on information processing and plasticity in MSNs.

In this chapter I have found that P_r plays a relatively small role in controlling the short-term plasticity of DA release. This suggests that the short-term plasticity of DA release is relatively stable in the face of perturbations of P_r, especially when nAChRs are active. Therefore the contrast between DA release evoked by burst vs. low frequency firing will remain fairly constant. The stability in this ratio may help maintain a consistent postsynaptic response to behaviourally evoked changes in DA neuron firing pattern, independent of changes in presynaptic fluctuations, such as changes in Ca^{2+} entry.

In contrast, nAChRs strongly control the short-term plasticity of DA release, not simply by changing P_r but by actively clamping release and causing strong, release-independent depression across all IPIs. This means that when ACh release is high, for example during the rebound excitation in ChIs following a reward-related pause in activity (Morris *et al.* 2004; Apicella *et al.* 2009; Apicella *et al.* 2011), there will be little contrast between DA signals evoked by burst vs. low frequency DA neuron firing. Additionally, this contrast will be completely unaffected by changes in P_r. Therefore, in these conditions, information carried by ChI firing, but not information carried by DA neuron firing pattern or presynaptic factors, will be transmitted to postsynaptic cells. In contrast, when nAChRs are not active, for example during the pause in ChI

firing (Morris *et al.* 2004; Apicella *et al.* 2009; Apicella *et al.* 2011), there will be a large contrast between DA signals evoked by different DA neuron firing patterns (Cragg 2006), which will be finely controlled by presynaptic factors which modulate P_r. Therefore in these conditions, information carried by DA neurons and DA presynaptic zones will be effectively transmitted to postsynaptic cells.

These findings may also have implications for the actions of the addictive drug nicotine. At concentrations found in smokers, nicotine is thought to desensitise nAChRs on DA terminals, and hence to inhibit nAChRs. The data presented in this chapter suggest that this will not only alter P_r, but will fundamentally shift the mode of operation of DA synapses, such that changes in DA neuron firing and presynaptic fluctuations in DA neurons will always be transmitted to postsynaptic cells, even when ChIs are active.

3.5 Summary

In this chapter it has been shown that P_r has only a subtle role in controlling the short-term plasticity of DA release, and only when nAChRs are not active, in contrast to the strong role of P_r observed in classical central synapses. nAChRs, however, greatly affect the short-term plasticity of DA release. This is not simply due to their effect on P_r as has been previously hypothesised; instead, nAChRs strongly clamp subsequent release and make it invariant with P_r, potentially by inducing action potential conduction failure. Therefore, nAChRs are much greater determinants of the short-term plasticity of DA release than presynaptic factors which simply modulate P_r. This has implications for understanding the downstream effects of changes in DA neuron and ChI firing on striatal DA signals, and for the effects of nicotine on information processing in the striatum.

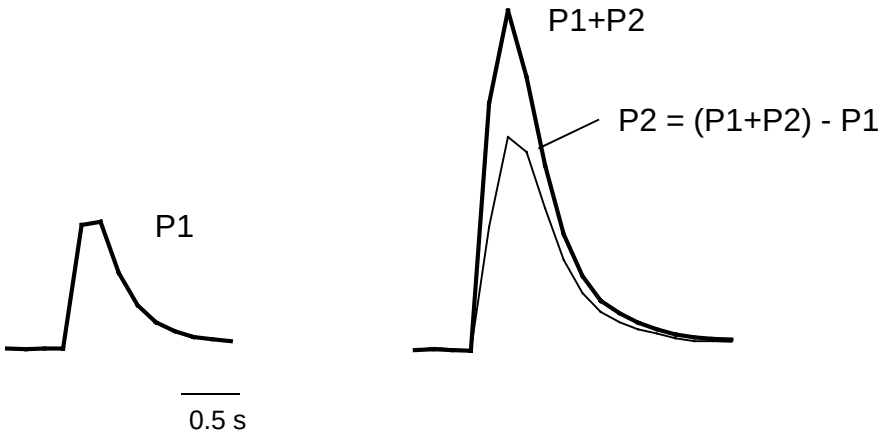


Figure 3.1. Determination of P2 for calculation of P2/P1. When two stimuli are applied with IPIs less than approximately 1 s, $[DA]_o$ evoked by the second pulse (P2) summates with $[DA]_o$ evoked by the first (P1). P2 is therefore determined by the subtraction of P1 from $[DA]_o$ evoked by two pulses (P1+P2).

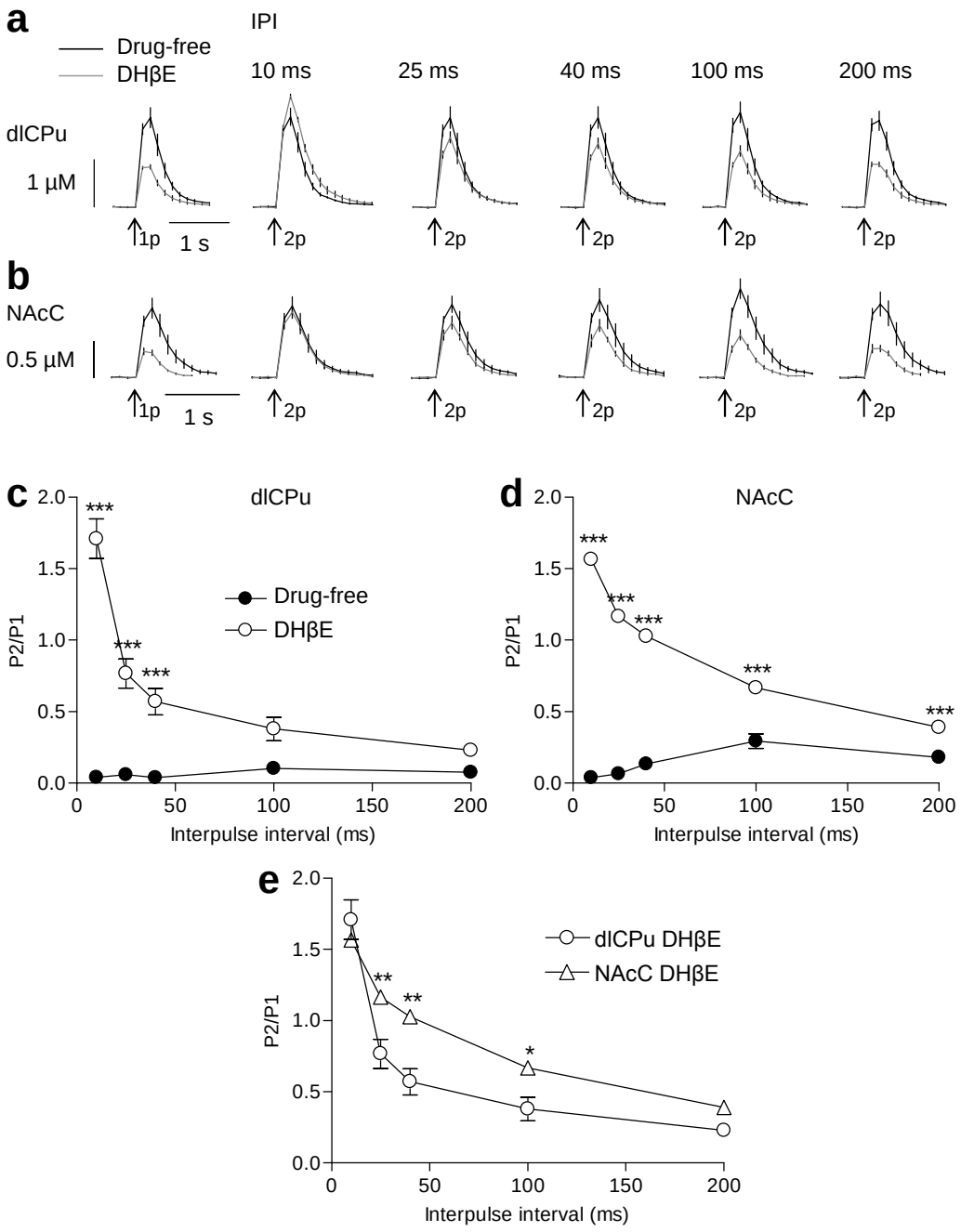


Figure 3.2 nAChRs strongly control the short-term plasticity of DA release. (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), in drug-free conditions, or with nAChR antagonist, DHβE (1 µM), in dlCPu (a) and NAcC (b). **(c,d)** Mean P2/P1±SEM against IPI in drug-free conditions and in DHβE (1 µM) in dlCPu (c) and NAcC (d). DHβE significantly increased P2/P1 at 10-40 ms in dlCPu, and at all IPIs in NAcC (Two-way ANOVA, dlCPu: main effect DHβE $F_{1,20}$=232.92 *p < 0.001,* main effect IPI $F_{4,20}$=33.68 *p < 0.001*, interaction $F_{4,20}$=37.76 *p < 0.001*, *post hoc* Bonferroni t-test, *p < 0.001* at 10-40 ms IPI; *n = 3*; NAcC: main effect DHβE $F_{1,20}$=1622.12 *p < 0.001,* main effect IPI $F_{4,20}$=69.36 *p < 0.001*, interaction $F_{4,20}$=138.84 *p < 0.001*, *post hoc* Bonferroni t-test, *p < 0.001* at all IPIs; *n = 3*) **(e)** Mean P2/P1±SEM against IPI in DHβE (1 µM) in dlCPu and NAcC. P2/P1 was greater at 25-100 ms IPI in NAcC vs. dlCPu (Two-way ANOVA,main effect region $F_{1,20}$=26.58 *p < 0.001,* main effect IPI $F_{4,20}$=101.45 *p < 0.001*, interaction $F_{4,20}$=5.60 *p < 0.01*, *post hoc* Bonferroni t-test, *p < 0.05* at 25-100 ms IPI; *n = 3*).

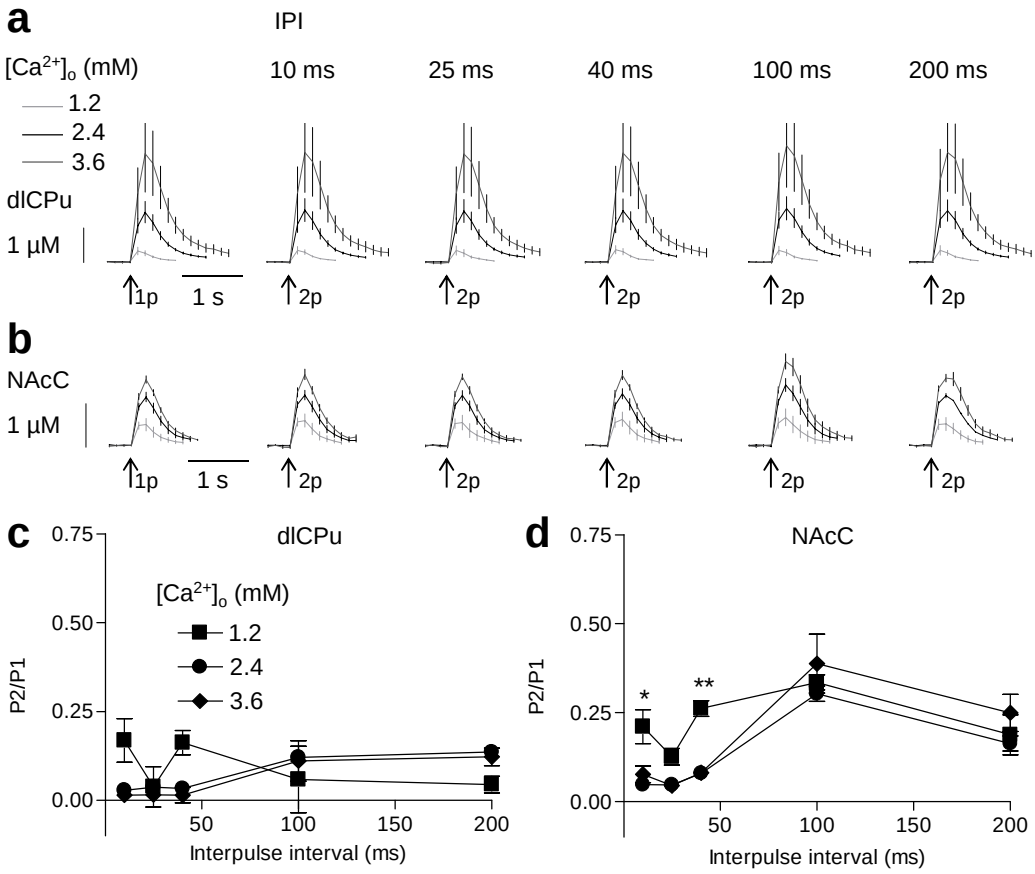


Figure 3.3 When nAChRs are active, changing P_r by changing $[Ca^{2+}]_o$ has little effect on the short-term plasticity of DA release. (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow) (1p or 2p, IPI 10-200 ms), in different $[Ca^{2+}]_o$ (1.2, 2.4 and 3.6 mM), in dlCPu (a) and NAcC (b). **(c,d)** Mean P2/P1±SEM against IPI in dlCPu (c) and NAcC (d), in different $[Ca^{2+}]_o$. In dlCPu, there was a significant interaction between $[Ca^{2+}]_o$ and IPI, but no significant effect at any given IPI (Two-way ANOVA, main effect $[Ca^{2+}]_o$ $p > 0.05$, $F_{2,30}$=1.25, main effect IPI $p > 0.05$, $F_{4,30}$=1.56, interaction $p < 0.05$, $F_{8,30}$=2.56, *post hoc* Bonferroni t-test, $p > 0.05$ at all IPIs; $n = 3$). In NAcC, P2/P1 was significantly increased at 10 and 40 ms IPI in 1.2 vs. 2.4 mM $[Ca^{2+}]_o$ but was not significantly altered in 3.6 vs. 2.4 mM $[Ca^{2+}]_o$ (Two-way ANOVA, main effect $[Ca^{2+}]_o$ $p < 0.001$, $F_{2,30}$=9.30, main effect IPI $p < 0.001$, $F_{4,30}$=26.40, interaction $p < 0.05$, $F_{8,30}$=2.55, *post hoc* Bonferroni t-test, $p < 0.05$ at 10 and 40 ms IPI in 1.2 vs. 2.4 mM $[Ca^{2+}]_o$; $n = 3$).

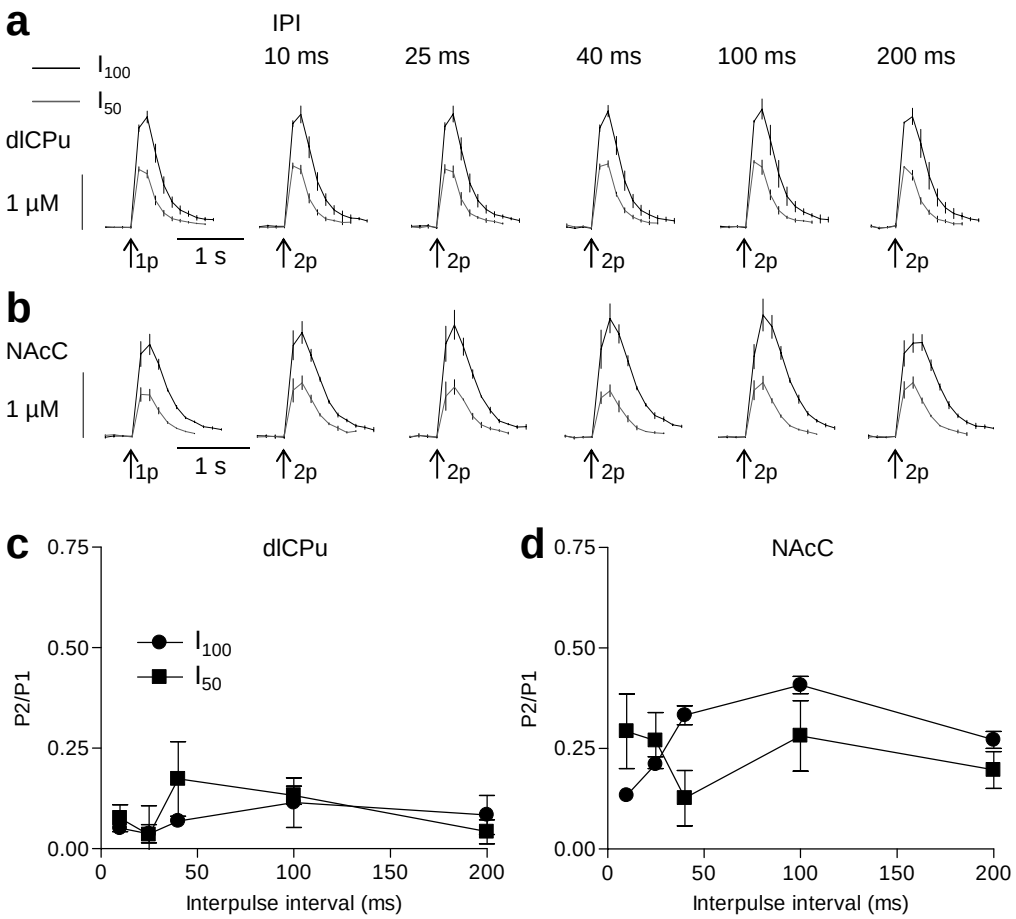


Figure 3.4 When nAChRs are active, changing P_r by changing stimulation current has little effect on the short-term plasticity of DA release. (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), with different stimulation currents (I_{100} or I_{50}), in dlCPu (a) and NAcC (b). **(c,d)** Mean P2/P1±SEM against IPI in dlCPu (c) and NAcC (d), with different stimulation currents. There was no significant effect of stimulation current on P2/P1in either region (Two-way ANOVA, dlCPu: main effect I $p > 0.05$, $F_{1,20}$=0.46, main effect IPI $p > 0.05$, $F_{4,20}$=1.31, interaction $p > 0.05$, $F_{4,20}$=0.62; NAcC: main effect I $p > 0.05$, $F_{1,80}$=1.20, main effect IPI $p > 0.05$, $F_{4,80}$=1.87, interaction $p > 0.05$, $F_{4,80}$=3.61; $n = 3$).

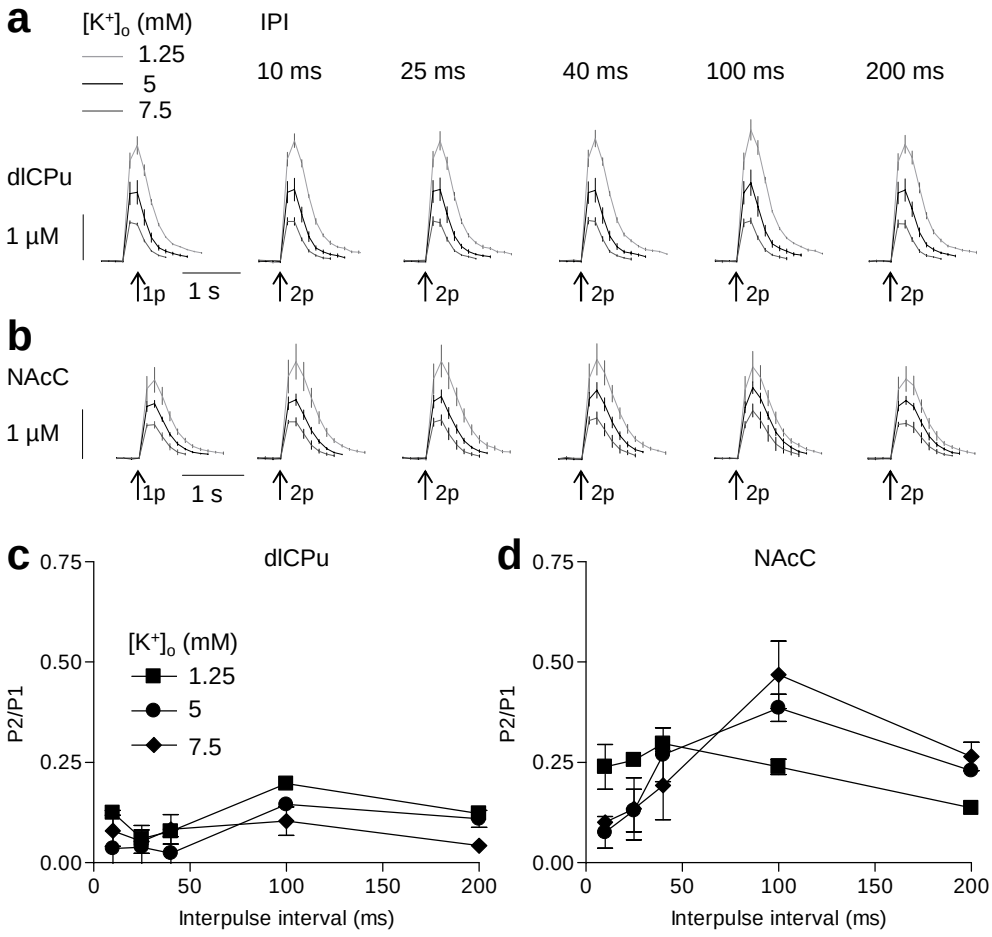


Figure 3.5 When nAChRs are active, changing P_r by changing $[K^+]_o$ has little effect on the short-term plasticity of DA release. (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), in different $[K^+]_o$ (1.25, 5 and 7.5 mM), in dlCPu (a) and NAcC (b). **(c,d)** Mean P2/P1±SEM against IPI in dlCPu (c) and NAcC (d), in different $[K^+]_o$. In dlCPu, there was no significant effect of $[K^+]_o$ on P2/P1 (Two-way ANOVA, main effect $[K^+]_o$ $p > 0.05$, $F_{2,30}$=2.48, main effect IPI $p < 0.05$, $F_{4,30}$=3.23, interaction $p > 0.05$, $F_{8,30}$=0.70; $n = 3$). In NACc, there was a significant interaction between $[K^+]_o$ and IPI but there was no significant effect of $[K^+]_o$ at any given IPI (Two-way ANOVA, main effect $[K^+]_o$ $p > 0.05$, $F_{2,30}$=0.15, main effect IPI $p < 0.001$, $F_{4,30}$=9.61, interaction $p < 0.01$, $F_{8,30}$=3.49, *post hoc* Bonferroni t-test, $p > 0.05$ at all IPIs; $n = 3$).

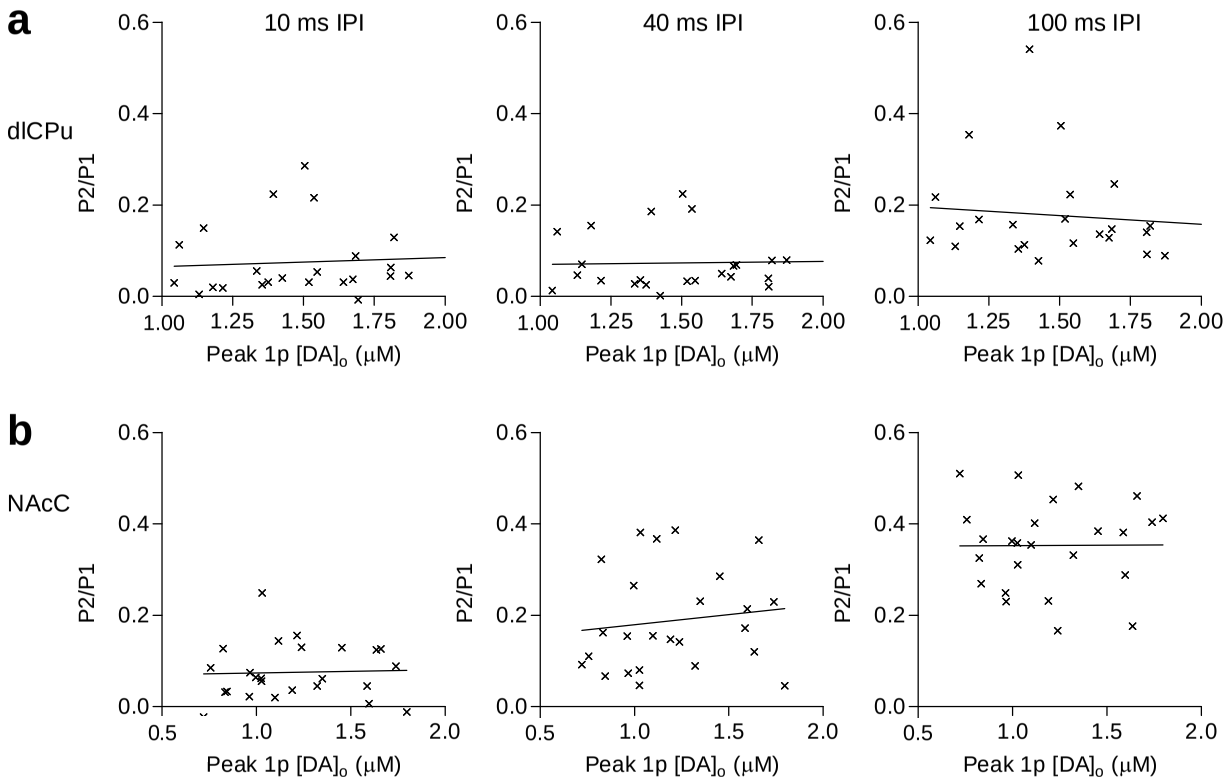


Figure 3.6 When nAChRs are active, there is no correlation between P2/P1 and peak $[DA]_o$ evoked by 1p. (a,b) P2/P1 at 10 (left), 40 (middle) and 100 (right) ms IPI against peak $[DA]_o$ evoked by 1p, at individual release sites, in dlCPu (a) and NAcC (b). There was no significant non-zero relationship between P2/P1 and peak $[DA]_o$ at any IPI in either region (linear regression analysis, $p > 0.05$, $0 < R^2 < 0.04$; $n = 25\text{-}35$).

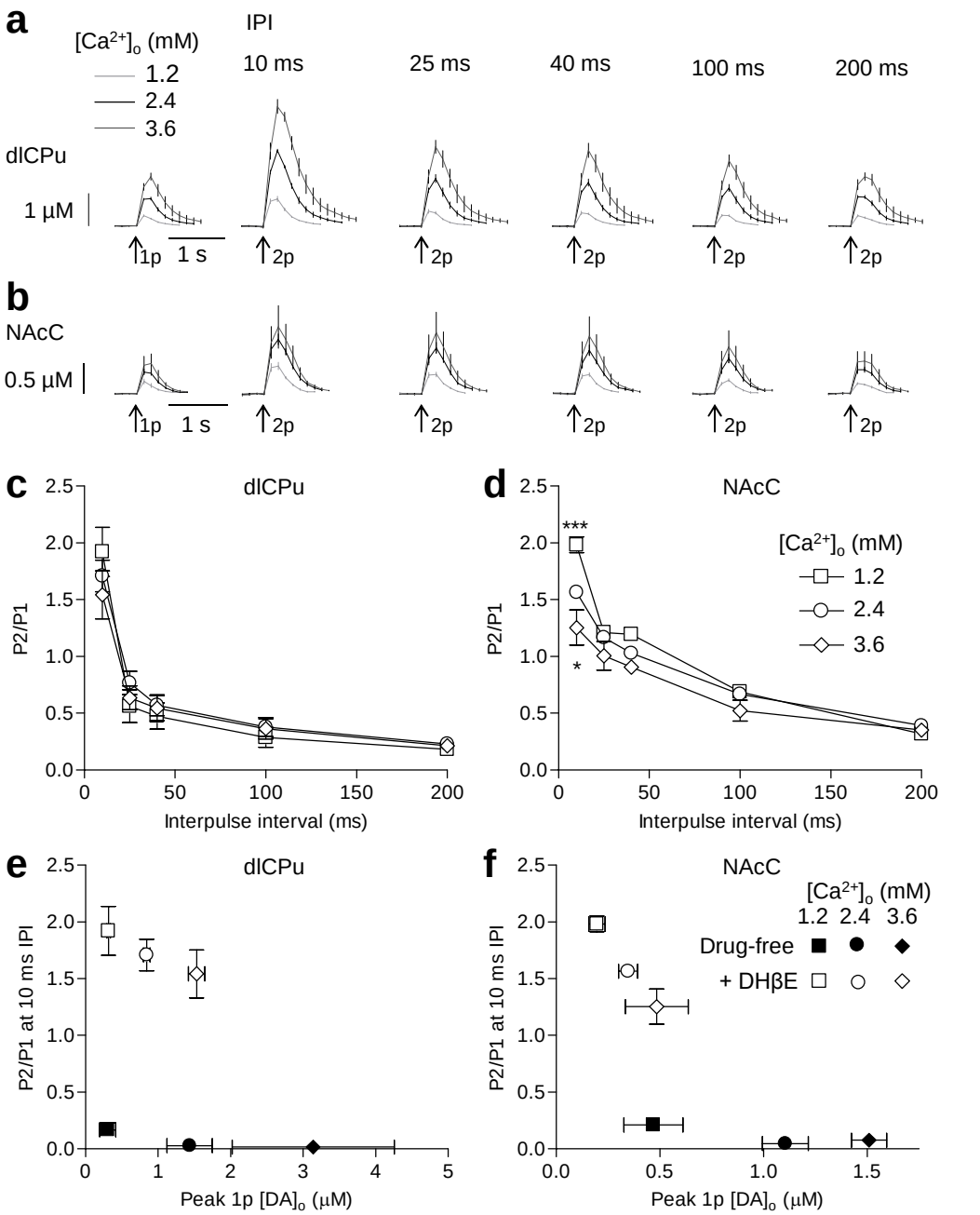


Figure 3.7 When nAChRs are inhibited, changing P_r by changing $[Ca^{2+}]_o$ has small effects on the short-term plasticity of DA release at short IPIs. (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), in different $[Ca^{2+}]_o$ (1.2, 2.4 and 3.6 mM), with nAChRs inhibited (DHβE, 1 µM), in dlCPu (a) and NAcC (b). **(c,d)** Mean P2/P1±SEM against IPI in different $[Ca^{2+}]_o$, with nAChRs inhibited (DHβE, 1 µM), in dlCPu (c) and NAcC (d). In dlCPu there was no significant effect of $[Ca^{2+}]_o$ on P2/P1 (Two-way ANOVA, main effect $[Ca^{2+}]_o$ $p > 0.05$, $F_{2,45}$=0.45, main effect IPI $p < 0.001$, $F_{4,45}$=76.13, interaction $p > 0.05$, $F_{8,45}$=0.80; $n = 4$). In NAcC, P2/P1 at 10 ms IPI was significantly increased in 1.2 vs. 2.4 mM $[Ca^{2+}]_o$ and decreased in 3.6 vs. 2.4 mM $[Ca^{2+}]_o$ (Two-way ANOVA, main effect $[Ca^{2+}]_o$ $p < 0.001$, $F_{2,30}$=19.97, main effect IPI $p < 0.001$, $F_{4,30}$=149.30, interaction $p < 0.01$, $F_{8,30}$=4.50, *post hoc* Bonferroni t-test, $p < 0.05$ at 10 ms IPI; $n = 3$). **(e,f)** Mean P2/P1 at 10 ms IPI ±SEM against mean peak $[DA]_o$±SEM evoked by 1p, in different $[Ca^{2+}]_o$, with and without nAChR inhibition, in dlCPu (e) and NAcC (f).

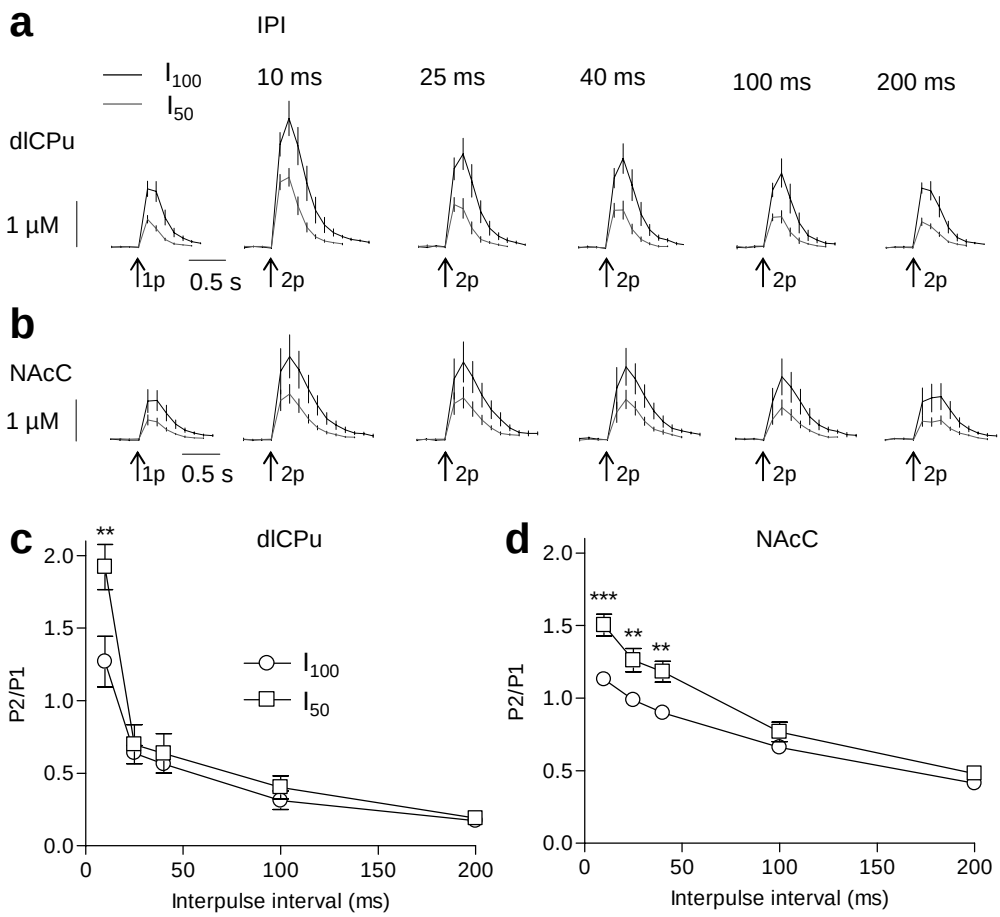


Figure 3.8 When nAChRs are inhibited, changing P_r by changing stimulation current has a small effect on the short-term plasticity of DA release. (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), with different stimulation currents (I_{100} or I_{50}), with nAChRs inhibited (DHβE, 1 µM), in dlCPu (a) and NAcC (b). **(c,d)** Mean P2/P1±SEM against IPI, with different stimulation currents, with nAChRs inhibited (DHβE, 1 µM), in dlCPu (c) and NAcC (d). In dlCPu, P2/P1 was significantly increased at 10 ms IPI at I_{50} vs. I_{100} (Two-way ANOVA, main effect I $p < 0.05$, $F_{1,20}$=7.50, main effect IPI $p < 0.001$, $F_{4,20}$=56.29, interaction $p < 0.05$, $F_{4,20}$=3.32, *post hoc* Bonferroni t-test, $p < 0.01$ at 10 ms IPI; $n = 3$). In NAcC, P2/P1 was significantly increased at 10-40 ms IPI at I_{50} vs. I_{100} (Two-way ANOVA, main effect I $p < 0.05$, $F_{1,80}$=44.71, main effect IPI $p < 0.001$, $F_{4,80}$=88.08, interaction $p < 0.05$, $F_{4,80}$=3.09, *post hoc* Bonferroni t-test, $p < 0.01$ at 10-40 ms IPI; $n = 3$).

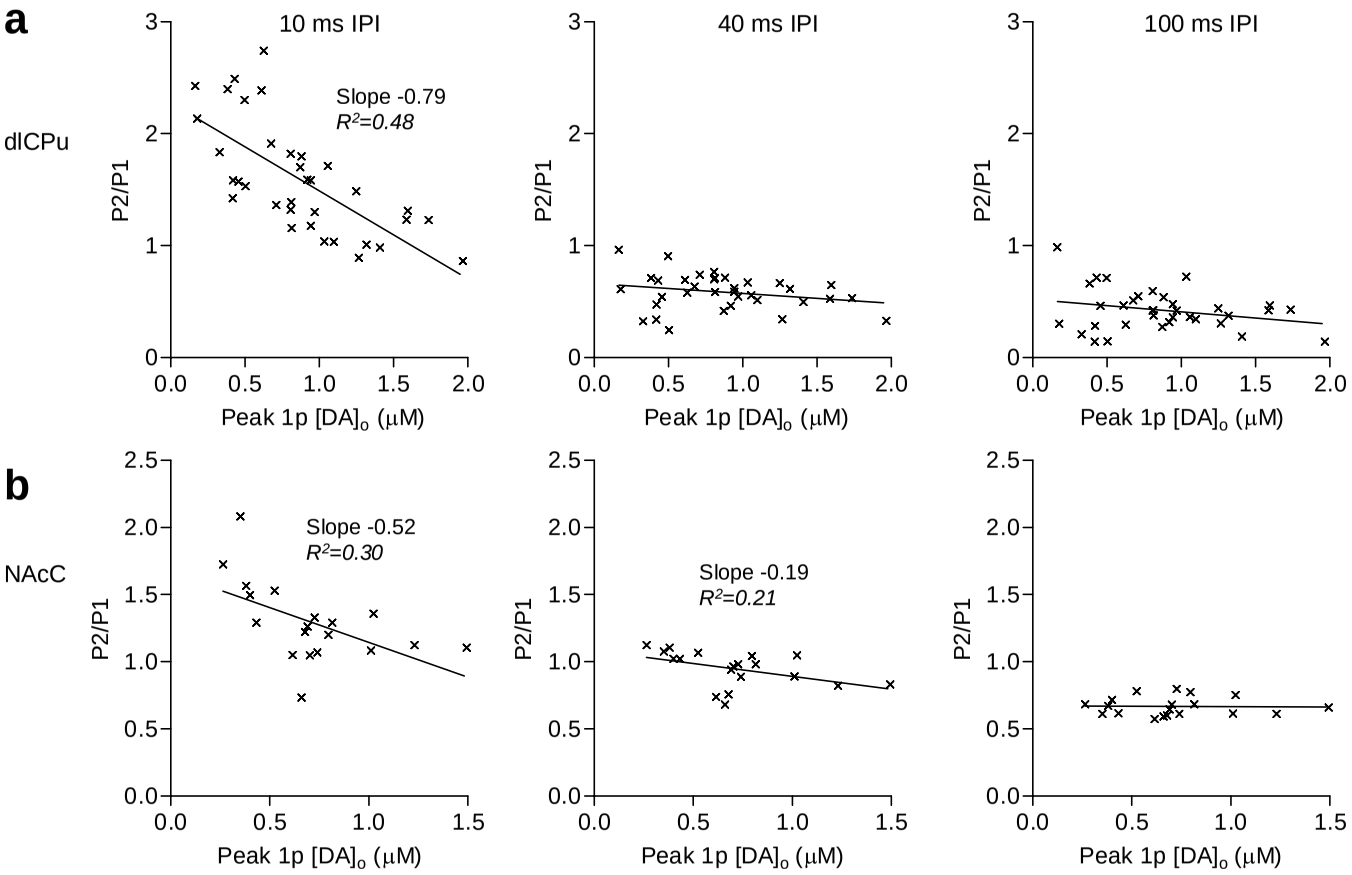


Figure 3.9 When nAChRs are inhibited, P2/P1 and peak $[DA]_o$ evoked by 1p are inversely correlated at short IPIs. (a,b) P2/P1 at 10 (left), 40 (middle) and 100 (right) ms IPI against peak $[DA]_o$ evoked by 1p at individual release sites, with DHβE, in dlCPu (a) and NAcC (b). In dlCPu, there was a significant inverse relationship between P2/P1 and $[DA]_o$ at 10 ms IPI (linear regression, *p<0.001*, slope = -0.79, *R^2=0.48*; *n = 35*) but no significant relationship at longer IPIs (linear regression, *p>0.05*, *0.06<R^2<0.07*; *n = 35*). In NAcC, there was a significant inverse relationship between P2/P1 and $[DA]_o$ at 10 and 40 ms IPI (linear regression, *p<0.05*, 10 ms: slope = -0.52, *R^2=0.30*; *n = 25*, 40 ms: slope = -0.19, *R^2=0.21*; *n = 25*) but no significant relationship at 100 ms IPI (linear regression, *p>0.05*, *R^2<0.01*; *n = 25*).

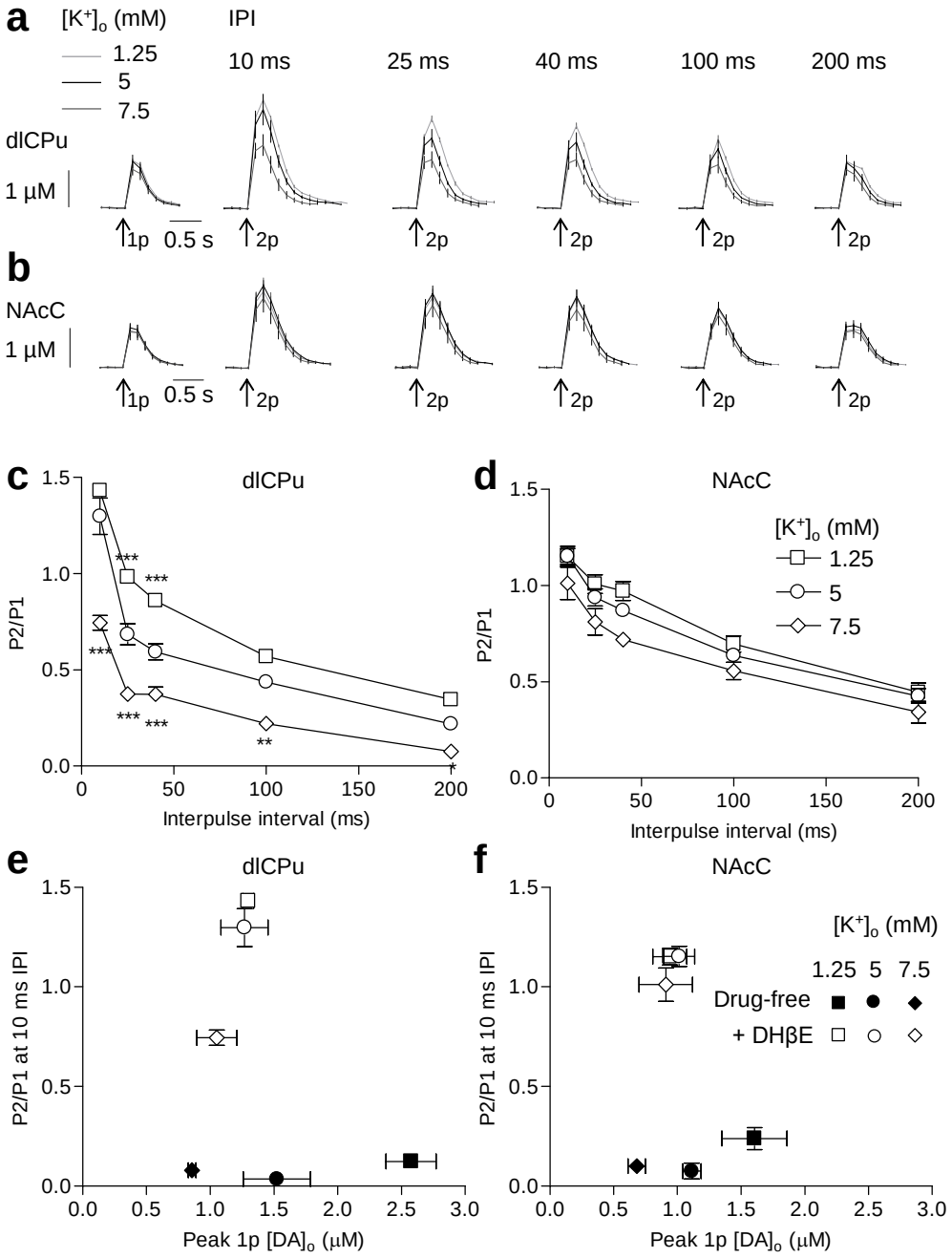


Figure 3.10 Changing $[K^+]_o$ demonstrates that the short-term plasticity of DA release and P_r are dissociable even when nAChRs are inhibited. (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), in different $[K^+]_o$ (1.25, 5 and 7.5 mM), with nAChRs inhibited (DHβE, 1 µM), in dlCPu (a) and NAcC (b). **(c,d)** Mean P2/P1±SEM against IPI in different $[K^+]_o$, with nAChRs inhibited (DHβE, 1 µM), in dlCPu (c) and NAcC (d). In dlCPu P2/P1 was significantly increased at 25 and 40 ms IPI in 1.25 vs. 5 mM $[K^+]_o$ and significantly decreased at all IPIs in 7.5 vs. 5 mM $[K^+]_o$ (Two-way ANOVA, main effect $[K^+]_o$ $p < 0.001$ $F_{3,70}$=151.05, main effect IPI $p < 0.001$, $F_{4,70}$=85.57, interaction $p < 0.001$, $F_{12,70}$=15.66, *post hoc* Bonferroni t-test, $p < 0.001$ at 25-40 ms IPI in 1.25 vs. 5 mM $[K^+]_o$ and 10-100 ms IPI in 7.5 vs. 5 mM $[K^+]_o$; $n = 3$). In NAcC there was a significant main effect of $[K^+]_o$ but no significant interaction between $[K^+]_o$ and IPI (Two-way ANOVA, main effect $[K^+]_o$ $p < 0.001$ $F_{2,30}$=15.15, main effect IPI $p < 0.001$, $F_{4,30}$=91.41, interaction $p > 0.05$, $F_{8,30}$=0.43; $n = 3$). **(e,f)** Mean P2/P1 at 10 ms IPI ±SEM against peak $[DA]_o$±SEM evoked by 1p, in different $[K^+]_o$, with and without nAChR inhibition, in dlCPu (e) and NAcC (f).

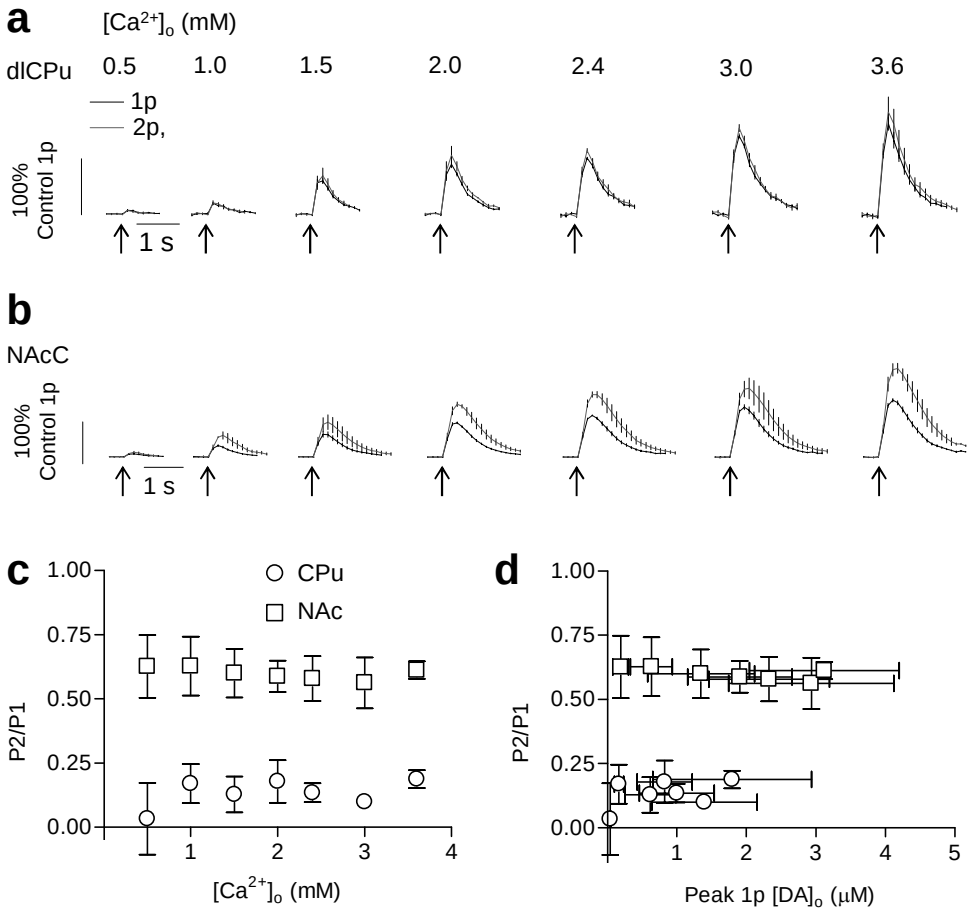


Figure 3.11 Using optogenetic stimulation of DA neurons, modulating P_r does not modulate the short-term plasticity of release at an IPI of 40 ms, in either striatal region. **(a,b)** Profiles of mean $[DA]_o$±SEM against time after single or paired pulse light stimulations (arrow) (1p or 2p, IPI 40 ms), in different $[Ca^{2+}]_o$ (0.5-3.6 mM), normalised to peak $[DA]_o$ evoked by 1p in 2.4 mM $[Ca^{2+}]_o$, in $DAT^{Cre/+}$ mice transfected with AAV5-ChR2, in dlCPu (a) and NAcC (b). **(c)** Mean P2/P1 at 40 ms IPI ±SEM against $[Ca^{2+}]_o$ in dlCPu and NAcC. **(d)** Mean P2/P1 at 40 ms IPI ±SEM against mean peak$[DA]_o$±SEM evoked by 1p, in dlCPu and NAcC.

4. The DAT directly controls the short-term plasticity of DA release when nAChRs are not active

4.1 Introduction

In this chapter I have explored a previously unidentified role for the DAT in controlling the short-term plasticity of DA release.

The DAT is an integral membrane protein with 12 transmembrane domains. It is a member of the solute carrier 6 (Slc6A), Na^+/Cl^- -dependent transporter family, which includes the other monoamine transporters, SERT and NET, as well as transporters for GABA and glycine(Torres *et al.* 2003). The DAT is a secondary active transporter. It uses the co-transport of Na^+ and Cl^- down their electrochemical gradients to move DA against its concentration gradient, from the extracellular space into the cell (Amejdki-Chab *et al.* 1992; Gu *et al.* 1994). The DAT is thought to co-transport two Na^+ ions and one Cl^- ion with every DA molecule (Kreuger 1990), and hence is electrogenic, generating a transport-coupled inward current. The DAT is expressed exclusively by DA neurons; unlike transporters for the classical neurotransmitters the DAT is not thought to be expressed in postsynaptic or glial cells (Freed *et al.* 1995). DAT density, and correspondingly the rate of uptake, varies across striatal regions, with the greatest density and uptake in CPu, followed by the NAcC, with lowest levels in NAc shell (Marshall *et al.* 1990; Jones *et al.* 1996; Nirenberg *et al.* 1997).

4.1.1 Functions of the DAT

The DAT has a complex function in controlling striatal DA signalling, composed of a number of aspects:

4.1.1.1 Recycling DA for re-release

The primary function of the DAT is to transport DA from the extracellular space into the presynaptic DA terminal, where it is re-packaged into vesicles ready for subsequent release. This is important for normal striatal DA homeostasis. Animals in which the DAT is deleted (DAT KO) show large changes in DA synthesis and turnover, for example TH expression is decreased by 90%

in these animals, but the rate of DA turnover is doubled (Jones SR 1997; Gainetdinov *et al.* 1998). Therefore, recycling of DA via the DAT, for repackaging and re-release, is crucial for normal DA homeostasis in the DA neuron terminal. However, the DAT also plays other important roles in the control of striatal DA signalling.

4.1.1.2 Shaping the extracellular DA signal

In the striatum, the DAT is the major mechanism for clearance of DA from the extracellular space and it therefore plays a significant role in shaping the extracellular DA signal. As described in chapter 1.1.3.1, models of striatal DA signalling suggest that the DAT does not function to 'gate' DA within the synaptic cleft, due to the subcellular location and slow turnover rate of the DAT; instead, DA is thought to spill over from the synapse by diffusion and hence act via 'volume transmission', as opposed to the point-to-point transmission of classical transmitters (Zoli *et al.* 1998; Cragg and Rice 2004; Arbuthnott and Wickens 2007). However, the DAT does limit the spatial and temporal extent of the DA signal, and hence its postsynaptic sphere of influence (Cragg and Rice 2004). This role is clearly demonstrated in DAT KO mice, in which the lifetime of $[DA]_o$ is 300 times that seen in WT animals (Giros *et al.* 1996; Jones SR 1997), and basal extracellular DA levels measured by microdialysis are increased fivefold (Jones SR 1997). DAT inhibitors also greatly increase the lifetime of $[DA]_o$ (Kelly and Wightman 1987). However, the role of the DAT in controlling extracellular DA is complex and dependent on a number of factors, including the size of the DA signal and the frequency of DA release. For example, DAT-mediated uptake more strongly limits DA signals evoked by low frequency firing, compared to release evoked by high frequency bursts of activity. This is due to the slow turnover rate of the DAT. During low frequency activity, the DAT is able to remove some DA from the extracellular space between release events. In contrast, during high frequency bursts, there is less time available for DA transport between release events, and so there is greater extracellular summation of DA signals (Garris *et al.* 1994; Garris and Rebec 2002; Cragg and Rice 2004). Therefore, the DAT plays

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presynaptic protein called synapsin, particularly the synapsin III isoform (Ewing *et al.* 1983; Venton *et al.* 2006; Kile *et al.* 2010).

4.1.2 DAT as a drug target

The DAT is the target of a number of addictive and therapeutic drugs. These fall into two main categories: DA releasers, such as amphetamines, which increase the intracellular concentration of DA, resulting in the reversal of the direction of the DAT and DA extrusion (Jones *et al.* 1998), and DAT inhibitors, such as cocaine, which block DA uptake via the DAT (Jones *et al.* 1995). It is well documented that the net effect of these drugs is to increase and prolong extracellular DA signals via their interactions with the DAT (Kelly and Wightman 1987; Wieczorek and Kruk 1994; Jones *et al.* 1995; Giros *et al.* 1996). This has numerous downstream effects in both the midbrain and the striatum, including causing changes in gene expression, changes in long-term synaptic plasticity and morphological changes in spine structure and density (Nestler 2001; Thomas *et al.* 2001; Ungless *et al.* 2001; Kourrich *et al.* 2007). These plastic changes will result in altered striatal output, and hence will disrupt reward-processing and action selection. In the cases of cocaine or amphetamine this can result in addiction, or for therapeutic drugs such as methylphenidate, used to treat ADHD, lead to therapeutic changes in behaviour.

However, as described above, the DAT plays a complex role in controlling extracellular DA, via multiple mechanisms. In order to fully understand how DAT inhibitor drugs alter striatal processing and behaviour, it is necessary to understand completely how the DAT, and hence DAT inhibition, modulates dopaminergic signalling.

4.1.3 DAT control of the short-term plasticity of DA release

Given that the DAT and DAT inhibition are thought to directly control DA release in addition to uptake (Stamford *et al.* 1989; Lee *et al.* 2001; Robinson and Wightman 2004; Venton *et al.* 2006), it may be hypothesised, as discussed in chapter 3.1, that they may also modulate the short-term

plasticity of DA release, particularly when nAChRs are not active. Additionally, it has previously been shown that the DAT may control some forms of DA release plasticity *in vivo*, in response to repeated prolonged stimulation (Chadchankar and Yavich 2011). Therefore in this chapter, I have investigated whether the DAT controls the short-term plasticity of striatal DA release, when nAChRs are inhibited. This will also have implications for understanding the actions of DAT inhibitors, like cocaine.

4.2 Methods

Methods were carried out as described in chapter 2, unless described otherwise here.

4.2.1 Animals

As described in chapter 2.1, the majority of experiments in this chapter were carried out using adult C57Bl6/J mice. Experiments investigating DA release in DAT^{Cre} mice used adult homozygote $DAT^{Cre/Cre}$ mice bred from animals as described in chapter 2.3.2. Experiments exploring release in animals with dopaminergic lesions used adult C57Bl6 male mice with unilateral 6-hydroxydopamine (6-OHDA) lesions. Adult male C57Bl6 mice were anaesthetized using isoflurane and placed in a stereotaxic frame. 7.5% marcaine was applied as a local anaesthetic and craniotomy was performed. 1 µl injections of 6-OHDA solution (4 µg/µl 6-OHDA plus 0.02 % acetic acid in 0.9 % saline) were given into the midbrain (co-ordinates from Bregma in mm: AP -3.5, ML ±1.0, DV -2.1) using a 33 gauge Hamilton syringe, at a rate of approximately 0.2 µl/min. After each injection the needle was left in place for 10 minutes and retracted slowly. The wound was then sutured using Ethilon monocryl non-absorbable suture. 0.5-0.7 ml 0.9% sterile saline was injected subcutaneously to replace lost fluids. Animals were maintained for 14-28 days post-surgery. Surgery was carried out by Dr Katie Jennings. Experiments exploring the role of synapsin III used adult male homozygote synapsin III knockout mice (S3KO) on a C57Bl6 background. These animals were bred from mice generated and described previously (Feng *et al.* 2002; Porton

et al. 2010), and were kindly supplied by Prof HT Kao (Brown University). All procedures were carried out in accordance with UK Home Office project licence and local guidelines.

4.2.2 Slice preparation and FCV

Striatal slices were prepared and FCV carried out as described in chapter 2.

In experiments exploring the effect of temperature, recording temperature was either reduced to 25^{o}C or increased to 37^{o}C. In all other experiments, the temperature of aCSF was maintained at approximately 32^{o}C throughout recordings.

Experiments in 6-OHDA lesioned animals were carried out in the ventromedial CPu, rather than dlCPu, since this was where $[DA]_o$ was above the threshold for detection.

In experiments exploring the effect of $[Ca^{2+}]_o$ or extracellular chloride concentration ($[Cl^-]_o$) , the composition of aCSF was modulated. Control aCSF contained in mM: 124.3 NaCl, 3.8 KCl and 2.4 $CaCl_2$ as described in chapter 2.2.2. Where $[Ca^{2+}]_o$ was reduced, aCSF contained 1.2 mM $CaCl_2$.Where $[Cl^-]_o$ was reduced, the following components differed from control, in mM: 57.9 NaCl, 66.4 Na-gluconate, 3.8 KCl and 2.4 $CaCl_2$. In experiments investigating $[DA]_o$ evoked by 1p in S3KO mice, aCSF contained 1.4 mM KCl, in order to replicate conditions used in a previous study (Kile 2010).

4.2.3 Electrical stimulation and experimental design

DA release was evoked using electrical stimulation as described in chapter 2.3.1. Unless described otherwise, all experiments in this chapter were carried out with nAChRs inhibited, using the β2* nAChR antagonist DHβE (1 μM), and therefore 'control conditions' are those with DHβE (1 μM) present.

Firstly, in experiments exploring the effect of DAT inhibition on the frequency response of $[DA]_o$, a single stimulus pulse (1p) or four pulse trains (4p, 5-100 Hz) were applied, in

pseudorandomized order and in triplicate. The ratio of peak $[DA]_o$ evoked by train stimulation to that evoked by 1p stimulation (4p:1p) was determined.

Secondly, in the majority of experiments in this chapter, a single stimulus pulse or pairs of pulses across all relevant IPIs (2p, 10-200 ms IPI), were applied, in pseudorandomized order and in triplicate. P2/P1 was determined as a measure of the short-term plasticity of DA release as described in chapter 3.2.3.

Thirdly, to explore the short-term plasticity of DA release on a background of activity two stimulation epochs were used. 1p or 2p (10-200 ms IPI) stimulations were applied at a 2nd stimulation epoch (E2), either 1.5 or 0.5 s after an initial (E1) 1p stimulation. E2 stimulations were applied in pseudorandomized order and in triplicate.

Finally, in experiments exploring the role of synapsin III, peak $[DA]_o$ evoked by 1p was explored throughout the striatum in S3KO vs. WT mice. This was to confirm previously reported findings from another lab (Kile *et al.* 2010). Therefore, in these experiments, the parameters of electrical stimulation used in that study (0.35 mA amplitude, 2 ms duration stimulation) were replicated. To obtain representative measurements of DA release, peak $[DA]_o$ evoked by 1p was measured in previously unstimulated recording sites in five sites per slice in CPu (dorsolateral, 1; dorsomedial, 2; ventrolateral, 3; ventromedial, 4; and central, 5;) and in two sites per slice in NAcC (lateral, 6; medial, 7) (**Fig. 4.1**) in drug-free conditions, in two slices per animal. Data was averaged across animals, within region. This protocol was then repeated with DHβE (1 μM) in one slice per animal.

4.2.4 Data analysis

Data were acquired and analysed as described in chapter 2.2.2. Comparisons of means were carried out in GraphPad Prism using Unpaired t tests, One- or Two-way ANOVAs and *post hoc* Bonferroni's t-tests, or in SPSS using Three-way ANOVAs. For analysis of DA uptake, the falling phases of $[DA]_o$ profiles evoked by 1p were curve fit using one phase exponential decay in

GraphPad Prism, *K* = rate constant of exponential decay. *n* = number of animals, except for experiments investigating $[DA]_o$ evoked by 1p throughout the striatum in S3KO vs. WT animals, where *n* = number of recording sites (Cragg 2003; Anwar *et al.* 2011), since $[DA]_o$ varies as much between striatal subregions within animals as between animals.

4.2.5 Drugs

DHβE, L-714,626 and lidocaine were purchased from Tocris Biosciences or Ascent Scientific. Cocaine hydrochloride and nomifensine maleate were purchased from Sigma-Aldrich and methylphenidate from Novartis. Drugs were dissolved in distilled water, aqueous acid (nomifensine maleate), ethanol (lidocaine) or dimethylsulphoxide (DMSO) (L-741,626) to make stock aliquots 1000-10000x final concentrations and stored at -20^{o}C. Final concentrations were made up in aCSF immediately before use.

4.3 Results

4.3.1 Inhibiting the DAT with cocaine alters the frequency response of $[DA]_o$

In this chapter I have explored a direct role for the DAT in controlling the short-term plasticity of DA release when nAChRs are not active. Unless described otherwise all experiments in this chapter have been carried out with nAChRs inhibited (DHβE, 1 µM) (called 'control conditions' throughout this chapter), to investigate the effects of the DAT on presynaptic DA release in the absence of the confounding effects of ACh.

To explore the effect of DAT inhibition on the frequency response of striatal DA signals, single pulse (1p) and four pulse train stimulations across a range of frequencies (4p, 5-100 Hz) were applied in recording sites in dlCPu and NAcC, before and after cocaine application (5 µM). As expected from the its role as a DAT inhibitor (Kelly and Wightman 1987; Wieczorek and Kruk 1994; Jones *et al.* 1995), cocaine increased the peak amplitude and duration of $[DA]_o$ profiles evoked by all stimulations in both striatal regions (**Fig. 4.2 a,b**). The ratio of peak $[DA]_o$ evoked by

4p vs. 1p stimulation (4p:1p) was determined at each frequency to explore the frequency sensitivity of $[DA]_o$. In control conditions, as previously shown (Rice and Cragg 2004; Zhang and Sulzer 2004), there was a positive relationship between 4p:1p and stimulation frequency, in both regions (**Fig. 4.2 c,d**). Cocaine significantly modified this relationship (Two-way ANOVA, $p < 0.001$; $n = 3$) (**Fig. 4.2 c,d**). Cocaine decreased the range of $[DA]_o$ evoked by different stimulation frequencies, decreasing the range of 4p:1p from a 3-4 fold range to a 2 fold range. Cocaine significantly increased 4p:1p at low frequencies (10 Hz in dlCPu, 5 Hz in NAcC, Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.01$; $n = 3$) but decreased it at high frequencies (100 Hz in dlCPu, 25-100 Hz in NAcC, Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.001$; $n = 3$). Therefore cocaine appears to reduce the frequency sensitivity of $[DA]_o$.

4.3.2 Inhibiting the DAT with cocaine alters the short-term plasticity of DA release

$[DA]_o$ is a compound measure of DA release and uptake. The reduction in the frequency sensitivity of $[DA]_o$ with cocaine may therefore be due either to changes in the extracellular summation of DA due to reduced uptake, or changes in DA release itself. Due to the different effects of the DAT on the extracellular summation of DA signals evoked by low and high frequency activity, as described above in 4.1.1.2 (Garris *et al.* 1994; Garris and Rebec 2002), DAT inhibition preferentially increases extracellular summation of DA signals evoked by low frequency activity. Hence this effect of DAT inhibition on summation, rather than any change in the plasticity of DA release, may underlie the effects of cocaine on 4p:1p at low frequencies. However, such changes in extracellular summation cannot readily account for the decrease in 4p:1p at high frequencies, suggesting that cocaine has an additional or alternative effect on DA signalling. To investigate whether cocaine affects the short-term plasticity of DA *release*, in addition to its effects on uptake, paired-pulse stimulations with IPIs across the relevant range (10-200 ms) were applied and P2/P1 determined, before and after cocaine application. Peak values of P1 and P2 should be approximately equally influenced by DA uptake. Therefore, in

contrast to net $[DA]_o$, or 4p:1p, P2/P1 should be independent of the extracellular summation of DA signals. Therefore P2/P1 can be used as a measure of the short-term plasticity of DA release, independent of uptake (Cragg 2003).

As expected, cocaine increased the peak amplitude and duration of DA profiles evoked by all stimulations, in both striatal regions (**Fig. 4.3 a,b**). The effect of cocaine on uptake was quantified by analysing the falling phases of DA profiles evoked by 1p, which are a function of DA uptake (Giros 1996) (**Fig. 4.3 c,d**). The falling phases were curve fit using one phase exponential decays. The rate of decay was significantly increased by cocaine in both regions (dlCPu: K=3.28 s^{-1} control, K=0.97 s^{-1} in cocaine, $p < 0.0001$; $n = 4$, $R^2 > 0.97$; NAcC: K=3.04 s^{-1} control, K=0.76 s^{-1} in cocaine, $p < 0.0001$; $n = 4$, $R^2 > 0.91$).

In control conditions, there was an inverse relationship between P2/P1 and IPI in both regions (**Fig. 4.3 e,f**). Cocaine significantly reduced this relationship in both regions, decreasing P2/P1 at short IPIs (10 ms in dlCPu, 10-25 ms in NAcC, Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.05$; $n = 4$) and increasing it at 25-40 ms IPIs in dlCPu (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.05$; $n=4$) with a trend towards an increased P2/P1 at longer IPIs in dlCPu (**Fig. 4.3 e,f**). Therefore, cocaine changes the short-term plasticity of DA release, reducing the frequency sensitivity of DA release. This suggests that the effects of cocaine on the frequency sensitivity of $[DA]_o$ are at least partly due to changes in the short-term plasticity of DA release, in addition to changes in extracellular DA summation.

4.3.3 Inhibiting the DAT using other DAT inhibitors alters the short-term plasticity of DA release

Cocaine is a fairly unspecific drug and has a number of high-affinity targets in addition to the DAT. For example cocaine is able to inhibit other monoamine uptake transporters including the SERT and NET, inhibit VGSCs (Steffensen *et al.* 2008) and bind sigma receptors (Sharkey *et al.* 1988). In order to investigate whether the effects of cocaine on the short-term plasticity of DA

release are due to the inhibition of the DAT or other effects of cocaine, the effects of two other DAT inhibitors and the local anaesthetic lidocaine on the short-term plasticity of DA release have been explored.

The effects of the DAT inhibitor methylphenidate on the short-term plasticity of DA release were explored. A paired-pulse stimulation procotol was applied in both dlCPu and NAcC, before and after methylphenidate (5 µM) application. As expected from its role as a DAT inhibitor, methylphenidate increased the peak amplitude and duration of DA profiles evoked by all stimulations in both regions (**Fig. 4.4 a,b**). In addition, the rate of decay of DA profiles evoked by 1p was significantly reduced by methylphenidate (dlCPu: K=2.75 s^{-1} control, K=0.73 s^{-1} methylphenidate $p < 0.0001$; $n = 5$, $R^2 > 0.94$; NAcC: K=4.20 s^{-1} control, K=0.57 s^{-1} methylphenidate, $p < 0.0001$; $n = 3$, $R^2 > 0.92$) (**Fig. 4.4 c,d**). Methylphenidate had a similar effect to cocaine on P2/P1. Methylphenidate reduced the inverse relationship between P2/P1 and IPI, reducing P2/P1 at 10 ms IPI in both regions (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.001$; $n = 3$-5) but tending to increase P2/P1 at longer IPIs in dlCPu (**Fig. 4.4 e,f**).

Since the gross effect of DAT inhibition on the relationship between P2/P1 and IPI appears similar in dlCPu and NAcC, the mechanism of this effect has been largely explored in dlCPu only from here onwards. Next, the effect of the DAT and NET inhibitor nomifensine (10 µM) was explored. Similarly to methylphenidate and cocaine, nomifensine increased the peak amplitude and duration of DA profiles across all stimulations (**Fig. 4.5 a**). It also significantly decreased the rate of decay of DA profiles evoked by 1p (**Fig. 4.5 b**) (K=3.65 s^{-1} control, K=0.37 s^{-1} nomifensine, $p < 0.0001$; $n = 5$, $R^2 > 0.92$). Again nomifensine diminished the relationship between P2/P1 and IPI, decreasing P2/P1 at 10 ms IPI (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.01$; $n = 5$) and tending to increase it at longer IPIs (**Fig 4.5 c**).

One well-characterised, non-DAT-mediated effect of cocaine is to inhibit VGSCs and hence function as a local anaesthetic. Since VGSCs play a key role in generating neuronal action

potentials, and hence in controlling transmitter release, it is possible that cocaine may alter the short-term plasticity of DA release via modulation of Na^+ currents rather than DAT inhibition. This is unlikely though as cocaine has a much greater potency as a DAT inhibitor than a VGSC inhibitor, and the concentration of cocaine used above is thought to have only very weak effects on VGSCs (Gifford and Johnson 1992; Hill *et al.* 2009). To rule out a role for VGSC inhibition in the effects of cocaine on DA release, the effects on the short-term plasticity of DA release of the related local anaesthetic lidocaine, which inhibits VGSCs with high potency but has extremely low affinity for the DAT (Woodward *et al.* 1995), were explored. In contrast to the DAT inhibitors, lidocaine (5 μM) decreased the peak amplitude of DA profiles, across all stimulations (**Fig. 4.6 a**), consistent with its role inhibiting VGSCs. Consistent with its very low affinity for the DAT, lidocaine had no effect on the rate of decay of DA profiles evoked by 1p ($p > 0.05$, $n = 3$; $R^2 > 0.87$) (**Fig. 4.6 b**). Furthermore, lidocaine had no effect on P2/P1 (Two-way ANOVA, $p > 0.05$; $n = 3$) (**Fig. 4.6 c**). These results suggest that any local anaesthetic properties of cocaine do not play a role in its effects on the short-term plasticity of DA release.

Therefore all tested DAT inhibitors, but not lidocaine, had similar effects on the short-term plasticity of DA release, decreasing the frequency sensitivity of release. This suggests that DAT inhibition, as opposed to any other effects of cocaine, control the short-term plasticity of DA release.

4.3.4 Endogenous DAT activity controls the short-term plasticity of DA release

The above results suggest that DAT inhibition alters the short-term plasticity of DA release. However these experiments do not directly address the question of whether the physiological activity of the DAT, as opposed to pharmacological DAT inhibition, controls the short-term plasticity of DA release. Here I have explored a role for endogenous DAT activity in controlling the short-term plasticity of DA release in three ways: using $DAT^{Cre/Cre}$ mice, using mice with a 6-OHDA lesion, and decreasing experimental temperature.

$DAT^{Cre/Cre}$ mice are engineered to express Cre-recombinase under control of the DAT promoter using a knockin approach. In this mouse line an internal ribosome entry site has been used to drive Cre-recombinase expression from the 3' untranslated region of the DAT gene to minimise changes in DAT expression. Despite this, striatal DAT protein levels are decreased by approximately 40-50% in $DAT^{Cre/Cre}$ mice (Bäckman *et al.* 2006). Therefore these animals have been used as a model to investigate the effect of decreased DAT expression on the short-term plasticity of DA release. The peak amplitude and duration of DA profiles evoked by all stimulations were increased in $DAT^{Cre/Cre}$ vs. WT (**Fig. 4.7 a,b**), as might be expected from their reduced DAT expression. In these experiments different CFMs were used between genotypes. CFMs can vary in their kinetics. Therefore here, in contrast to experiments where conditions were varied within an experiment using a single CFM, care must be taken in extrapolating from the comparisons of DA uptake between genotypes. In dlCPu, the rate of decay of DA profiles evoked by 1p was not different between genotypes ($p > 0.05$, $n = 3$; $R^2 > 0.91$) (**Fig. 4.7 c**) but in NAcC the rate of decay of DA profiles evoked by 1p was significantly slower in $DAT^{Cre/Cre}$ versus WT (K=2.40 s^{-1} WT, K=1.43 s^{-1} $DAT^{Cre/Cre}$, $p < 0.0001$; $n = 3$, $R^2 > 0.90$) (**Fig. 4.7 d**). In both regions the relationship between P2/P1 and IPI was significantly reduced in $DAT^{Cre/Cre}$ compared to WT, with P2/P1 lower at short IPIs in $DAT^{Cre/Cre}$ versus WTs (10 ms in dlCPu, 10-25 ms in NAcC, Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.05$; $n = 3$) (**Fig. 4.7 e,f**). These results support a physiological role for the DAT in the control of the short-term plasticity of DA release at short IPIs. However $DAT^{Cre/Cre}$ mice did not show the increase in P2/P1 at longer IPIs seen in dlCPu with pharmacological DAT inhibition.

To further investigate a physiological role for the DAT in controlling the short-term plasticity of DA release, the relationship between P2/P1 and IPI was explored in animals which had received partial unilateral 6-OHDA lesions to the midbrain. 6-OHDA causes the degeneration of nigrostriatal DA neurons, by generating increased oxidative stress in these cells. It is well-characterised that DAT-mediated DA uptake is reduced in such lesion models due to the passive

loss of DA terminals (Garris *et al.* 1997; Bergstrom and Garris 2003) and possibly a further compensatory decrease in DA uptake in remaining terminals (Bezard *et al.* 2000). These experiments were carried out in ventromedial CPu, since $[DA]_o$ was above the limit of detection here but not in dlCPu. In 6-OHDA lesioned animals, the peak amplitude of DA profiles was reduced across all stimulations in the hemisphere ipsilateral to the lesion vs. the contralateral hemisphere (**Fig. 4.8 a**). The rate of decay of DA profiles evoked by 1p was significantly reduced in the ipsilateral vs. contralateral hemisphere (K=2.55 s^{-1} contralateral, K=2.07 s^{-1} ipsilateral, $p < 0.005$; $n = 3$, $R^2 > 0.92$) (**Fig. 4.8 b**), consistent with reduced DAT activity in this model (Garris *et al.* 1997; Bezard *et al.* 2000; Bergstrom and Garris 2003). The 6-OHDA lesion also significantly altered the short-term plasticity of DA release; P2/P1 was significantly reduced at 10 ms IPI in the ipsilateral compared to the contralateral hemisphere (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.001$; $n = 3$) (**Fig. 4.8 c**). Therefore 6-OHDA lesions, which reduce DA uptake, have similar effects to DAT inhibition on the short-term plasticity of DA release.

The effect of decreasing experimental temperature on the short-term plasticity of DA release was also explored. Decreasing temperature should slow all enzymatic or stochastic processes within the DA terminal, including DAT-mediated transport. Reducing temperature from 32°C to 25°C reduced the peak amplitude of DA profiles across stimulations (**Fig. 4.9 a**). It also slightly reduced the rate of decay of DA profiles evoked by 1p (K=3.71 s^{-1} 32°C, K=3.25 s^{-1} 25°C, $p < 0.05$; $n = 3$, $R^2 > 0.95$) (**Fig. 4.9 b**). The short-term plasticity of DA release was significantly modulated by the decrease in temperature, with P2/P1 decreased at 10 ms IPI but increased at 25 ms IPI at 25 vs. 32°C (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.05$; $n = 3$) (**Fig. 4.9 c**). Therefore decreasing temperature, which decreases DA uptake, mimics the effects of DAT inhibition on the short-term plasticity of DA release.

Together these results support a physiological role for the DAT in controlling the short-term plasticity of DA release, promoting the frequency sensitivity of DA release.

4.3.5 DAT effects on the short-term plasticity of DA release do not depend on DA uptake via the DAT

As described above in 4.1, the DAT has a number of functions in the DA neuron terminal. I have begun to explore which action of the DAT may underlie the control of the short-term plasticity of DA release.

I have explored first whether the transport of DA via the DAT is central to the control of the short-term plasticity of DA release. In the above experiments, DA transport via the DAT is predicted to be low at the time of stimulation, since there has been a 2.5 minute interval since previous DA release. The DAT-mediated control of the short-term plasticity of DA release would therefore seem to be operating independently of the availability, and transport, of DA itself. Here I have explored whether the ongoing translocation of DA by the DAT modifies this effect. The short-term plasticity of DA release was explored at a 2nd epoch (E2) stimulation, that is a stimulation a few seconds after an initial (1st epoch, E1) stimulation. At this E2 time point, DA released by E1 will still be being transported by the DAT. Therefore, DA transport via the DAT is predicted to be higher at E2 than at E1. If the effect of the DAT on the short-term plasticity of DA release does depend on DA translocation via the DAT, the short-term plasticity of DA release should be different at E1 and E2, in a manner that is abolished by DAT inhibition.

DA transport via the DAT is greatest at very short time intervals after E1, but it is also more difficult to resolve peak $[DA]_o$ evoked by E1 and E2 with very short intervals between the epochs. To offset these two factors, two different intervals between E1 and E2 were used: 1.5 and 0.5 s. These experiments were carried out in the presence of the D_2R antagonist, L-741,626 (1 µM) to remove any confounding effects of autoinhibition which may be significant at E2 (Benoit-Marand *et al.* 2001; Schmitz *et al.* 2002). D_2R inhibition alone had no effect on the short-term plasticity of DA release in cocaine (Two-way ANOVA, $p > 0.05$; $n = 3$) (**Fig. 4.11 g**).

At both E1 and E2, with both a 1.5 and 0.5 s interval, cocaine increased the amplitude and duration of DA profiles evoked by all stimulations (**Fig. 4.10, 4.11 a,b**). At E1, as described above, cocaine significantly reduced the relationship between P2/P1 and IPI, reducing P2/P1 at 10 ms IPI (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.001$; $n = 3$) with a trend towards increased P2/P1 at longer IPIs (**Fig. 4.10, 4.11 c**). Cocaine modulated the relationship between P2/P1 and IPI in a similar manner at E2 (**Fig. 4.10, 4.11 d**).

To explore whether the short-term plasticity of DA release was altered by increased DA translocation, P2/P1 was compared at E1 and E2 (**Fig. 4.10, 4.11 e**). In control conditions, P2/P1 was higher at E2 than E1 across IPIs, particularly with a 1.5 s interval (1.5 s interval, Two-way ANOVA, main effect of epoch, $p < 0.01$, but no significant interaction between epoch and IPI, $p > 0.05$; $n = 3$) (**Fig. 4.10 e**). Therefore the short-term plasticity of DA release is slightly different at E2 compared to E1. To explore whether this effect is dependent on the DAT, P2/P1 was compared at E2 vs. E1 with cocaine. With cocaine, P2/P1 was also increased at E2 vs. E1 at all IPIs (Two-way ANOVA, main effect of epoch, $p < 0.001$; $n = 3$) (**Fig. 4.10, 4.11 f**). Therefore, since the effect of epoch on P2/P1 is similar with appear to depend on the DAT. These results suggest that there is no effect of increased DA transport via the DAT on the short-term plasticity of DA release, and hence that the effect of the DAT on the short-term plasticity of DA release is independent of DA translocation.

4.3.6 DAT effects on the short-term plasticity of DA release at short and long IPIs are dissociable

As described above in 4.3.2, the DAT appears to have two effects on the short-term plasticity of DA release over 10-200 ms in dlCPu. DAT inhibition reduces P2/P1 at short IPIs (10 ms) and tends to increase it at longer IPIs (25-100 ms). In contrast in NAcC, DAT inhibition appears only to decrease P2/P1 at short IPIs (10-40 ms), with little effect at longer IPIs (see **Fig. 4.3 e,f**). It is thus far uncertain whether these effects over different time courses in dlCPu represent one action,

modulating the overall relationship between P2/P1 and IPI, or two distinct actions with different time courses, one increasing and one decreasing P2/P1. DAT inhibition is known to increase DA release (Venton *et al.* 2006), and the results presented in chapter 3 suggest that this may result in a decrease in P2/P1 at very short IPIs but have very little effect at longer IPIs. Therefore it is feasible that DAT inhibition has two distinct effects on release plasticity, one at very short IPIs due to the increase in initial release, and one at longer IPIs. Here, different concentrations of cocaine were used to try and dissect these two effects of DAT inhibition.

Both a low (100 nM) and high (5 µM) concentration of cocaine altered the shape of DA profiles (**Fig. 4.12 a**). The rate of decay of DA profiles evoked by 1p was slightly decreased by 100 nM cocaine and decreased further by 5 µM (K=7.39 s^{-1} control, K=5.63 s^{-1} cocaine (100 nM),K=1.38 s^{-1} cocaine (5 µM), $p<0.0001$; $R^2>0.94$ $n = 3$) (**Fig. 4.12 b**). However, 100 nM cocaine had no significant effect on peak $[DA]_o$ evoked by 1p (One-way ANOVA, *post hoc* Bonferroni t test, $p > 0.05$; $n = 3$), whereas 5 µM significantly increased peak $[DA]_o$ evoked by 1p (One-way ANOVA, *post hoc* Bonferroni t test, $p<0.001$; $n = 3$) (**Fig. 4.12 c**). Therefore, this low concentration of cocaine significantly slowed uptake but its effects on DA release were not significant.

100 nM cocaine significantly modulated the relationship between P2/P1 and IPI, increasing P2/P1 at 25 IPI (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.05$; $n = 3$) (**Fig. 4.12 d**). However this low concentration had no effect on P2/P1 at 10 ms IPI (Two-way ANOVA, *post hoc* Bonferroni t test, $p > 0.05$; $n = 3$). In contrast, as shown above, 5 µM cocaine greatly decreased P2/P1 at 10 ms IPI (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.001$; $n = 3$) (**Fig. 4.12 d**).

These results demonstrate that the two effects of DAT inhibition on the short-term plasticity of DA release in dlCPu, at short and longer IPIs respectively, are dissociable, since 100 nM cocaine increased P2/P1 at longer IPIs but had no effect at short IPIs. Also, since this low concentration of cocaine reduced DA uptake but did not significantly increase peak $[DA]_o$, these results hint that

the effect of DAT inhibition at longer IPIs may depend on the inhibition of DA uptake, whereas the effect at short IPIs may depend on the increase in initial DA release.

4.3.7 DAT effects on the short-term plasticity of DA release are independent of synapsin III

The effect of DAT inhibition to increase DA release is reported to be dependent on the presynaptic protein synapsin (Venton *et al.* 2006). This is thought to involve the synapsin III isoform since this is believed to be the only synapsin family member expressed in DA neuron terminals (Pieribone *et al.* 2002; Bogen *et al.* 2006) and mice in which the gene encoding synapsin III has been disrupted (S3KO) show similar changes in striatal DA release compared to mice in which all three synapsin genes have been deleted (Kile *et al.* 2010). Since the above results hinted that the effect of DAT inhibition on the short-term plasticity of DA release at short IPIs may depend on the increase in DA release seen with DAT inhibition, I have explored whether the effects of the DAT on the short-term plasticity of DA release are dependent on synapsin III, using S3KO mice.

I first wanted to confirm the reported differences in DA transmission in S3KO vs. WT mice. Kile *et al.* (2010) showed that peak striatal $[DA]_o$ evoked by 1p is increased in these animals, possibly due to reduced sequestration of DA vesicles in a reserve pool and hence increased availability for DA release. Therefore, $[DA]_o$ evoked by 1p was measured in S3KO vs. WT animals, in drug-free conditions (without nAChR inhibition) using the same experimental conditions used by Kile *et al* (see section 4.2). $[DA]_o$ can be highly variable between different striatal release sites. Therefore to obtain representative measurements of DA release, $[DA]_o$ was measured in five sites per slice in CPu and in two sites per slice in NAcC (**Fig. 4.1**). In CPu, peak $[DA]_o$ evoked by 1p was increased by approximately 23% in S3KO compared to WT animals (Unpaired t test, $p < 0.05$; $n = 60$) (**Fig. 4.13 a,b**), consistent with previous reports showing that $[DA]_o$ is increased in S3KOs (Kile 2010). In NAcC there was no significant difference between genotypes in peak $[DA]_o$ evoked by

1p (Unpaired t test, *p>0.05*; *n = 24*) although there was a trend towards increased release in S3KO (**Fig. 4.13 a,b**). $[DA]_o$ was then measured in CPu with nAChRs inhibited (with DHβE, 1 μM), to ensure the effects of synapsin III knockout were due to changes in DA neuron terminals, not changes in ChIs and ACh release. There was no significant difference in peak $[DA]_o$ evoked by 1p between genotypes when nAChRs were inhibited (Unpaired t test, *p>0.05*; *n = 20*), although there was a trend towards increased $[DA]_o$ in S3KO, with the percentage difference between genotypes comparable to in drug-free conditions (**Fig. 4.13 a,b**). Consistent with previous reports (Kile *et al.* 2010), this suggests that at least part of the difference in DA release between S3KO and WT is due to the lack of synapsin III in DA terminals, rather than in ChIs.

The effects of cocaine on DA release and the short-term plasticity of release were then explored. Surprisingly, cocaine greatly increased both the peak amplitude and duration of DA profiles in S3KO mice (**Fig. 4.13 c,d**), in a very similar manner to WT, in both dlCPu and NAcC (compare **Figs. 4.13 c,d** and **4.3 a,b**). Indeed, the percentage increase in peak 1p $[DA]_o$ with cocaine was not different in WT vs.S3KO in either region (data from **4.3** and **4.13**, dlCPu: 147±20% WT, 198±62% S3KO; NAcC: 217±15% WT, 202±87% S3KO; Unpaired t test, *p > 0.05*; *n = 3-4*). This suggests that the lack of effect of cocaine on the amplitude of $[DA]_o$ which has been reported in triple synapsin knockout mice (Venton *et al.* 2006; Kile *et al.* 2010) is not in fact dependent on synapsin III as has been thought previously (Kile *et al.* 2010).

Cocaine also significantly modulated the short-term plasticity of DA release in S3KO mice in a similar manner to in WT mice, decreasing P2/P1 at 10 ms IPI in both regions and increasing P2/P1 at longer IPIs in dlCPu (Two-way ANOVA, *post hoc* Bonferroni t test, *p < 0.05*; *n = 3*) (**Fig. 4.13 e,f** compare with **Fig. 4.3 e,f**). Therefore, the effects of DAT inhibition on the short-term plasticity of DA release are not dependent on synapsin III, but this may be unsurprising given that the effects of DAT inhibition on peak $[DA]_o$ also appear to be independent of synapsin III.

4.3.8 DAT effects on the short-term plasticity of DA release at longer IPIs depend on $[Ca^{2+}]_o$

The synapsin-dependent increase in DA release seen with cocaine has been reported to be dependent on $[Ca^{2+}]_o$ (Kile *et al.* 2010). Therefore, to explore further whether this mechanism may be involved in the DAT control of the short-term plasticity of release, I have also explored whether the effects of the DAT on P2/P1 depend on $[Ca^{2+}]_o$.

As expected from its key role in transmitter release, and as shown in chapter 3, halving $[Ca^{2+}]_o$ reduced peak $[DA]_o$ evoked by all stimulations (**Fig. 4.14 a,b**). The peak amplitude and duration of DA profiles across all stimulations was increased in cocaine in both control and low $[Ca^{2+}]_o$ (**Fig. 4.14 a,b**). In both control and low $[Ca^{2+}]_o$, cocaine reduced the relationship between P2/P1 and IPI, decreasing P2/P1 at 10 ms IPI (Two-way ANOVA, *post hoc* Bonferroni t test, $p< 0.001$; $n = 3$) and, particularly in low $[Ca^{2+}]_o$, increasing it at longer IPIs (low $[Ca^{2+}]_o$ Two-way ANOVA, *post hoc* Bonferroni t test, $p< 0.001$; $n = 3$) (**Fig. 4.14 c,d**). Cocaine appeared to have a greater effect on P2/P1 at longer IPIs in low vs. control $[Ca^{2+}]_o$ (**Fig. 4.14 c,d**). A Three-way ANOVA showed that there was a significant interaction between $[Ca^{2+}]_o$, IPI and cocaine ($p < 0.05$; $n = 3$). This was therefore explored in more detail. In control conditions, halving $[Ca^{2+}]_o$ slightly but significantly increased P2/P1 at 10 ms IPI (Two-way ANOVA, *post hoc* Bonferroni t test, $p< 0.05$; $n = 3$) but had no effect at any other IPI (**Fig. 4.14 e**), consistent with its effect on P_r (see chapter 3). In contrast, in cocaine, halving $[Ca^{2+}]_o$ significantly increased P2/P1 at 10-100 ms IPI (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.001$; $n = 3$) (**Fig. 4.14 f**). Indeed, the percentage change in P2/P1 with cocaine was significantly increased at 40-100 ms IPI in low vs. control $[Ca^{2+}]_o$ (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.01$; $n = 3$) (**Fig. 4.14 g**). These results suggest that the effect of the DAT on the short-term plasticity of DA release in dlCPu at longer, but not shorter, IPIs depends on $[Ca^{2+}]_o$. This effect of $[Ca^{2+}]_o$ was not due to any effect of $[Ca^{2+}]_o$ on DA uptake, since the rate of decay of DA profiles evoked by 1p was unchanged by $[Ca^{2+}]_o$ ($p > 0.05$; $n = 3$, $R^2>0.84$) (**Fig. 4.14 h**).

4.3.9 Effects of $[Ca^{2+}]_o$ on the modulation of the short-term plasticity of DA release by DAT inhibition are not due to effects on P_r

Since changing $[Ca^{2+}]_o$ modulated the effects of DAT inhibition on the short-term plasticity of DA release and also modulates P_r, it may be that the effects of DAT inhibition depend on P_r rather than specifically on $[Ca^{2+}]_o$. To investigate this, the effect of changing stimulation current, which has been used in chapter 3 to modulate P_r in a similar manner to changing $[Ca^{2+}]_o$, has been explored.

A paired-pulse protocol was applied at a perimaximal current (I_{100}) and a current that approximately halved peak $[DA]_o$ evoked by 1p (I_{50}), before and after cocaine. Cocaine increased the peak amplitude and duration of DA profiles with both stimulation currents (**Fig. 4.15 a,b**). At both I_{100} and I_{50}, cocaine reduced the relationship between P2/P1 and IPI, decreasing P2/P1 at 10 ms IPI and increasing it at longer IPIs (Two-way ANOVA, *post hoc* Bonferroni t test, $p< 0.05$; $n = 3$) (**Fig. 4.15 c,d**). In contrast to changing $[Ca^{2+}]_o$, the effects of cocaine appeared similar between stimulation currents. This was explored further. In control conditions, changing stimulation current had no significant effect on P2/P1 (Two-way ANOVA, $p > 0.05$; $n = 3$) but showed a trend towards increasing P2/P1 at 10 ms IPI, reflecting the decrease in P_r (**Fig. 4.15 e**). Changing stimulation current also had no significant effect on P2/P1 in cocaine (Two-way ANOVA, $p > 0.05$; $n = 3$) (**Fig. 4.15 f**). A Three-way ANOVA also showed that there was no interaction between stimulation current, IPI and cocaine ($p > 0.05$; $n = 3$). Therefore, in contrast to changing $[Ca^{2+}]_o$, the effects of DAT inhibition on the short-term plasticity of DA release were not dependent on stimulation current. Therefore the effect of the DAT on the short-term plasticity of DA release depends specifically on $[Ca^{2+}]_o$, not just on P_r.

4.3.10 DAT effects on the short-term plasticity of DA release do not depend on DAT Cl^- conductance

In addition to recycling DA, the DAT shows ion channel-like conductances (Sonders 1997), which are capable of depolarizing DA cell bodies (Ingram *et al.* 2002), although their effect on DA neuron terminals is unexplored. Whilst the DAT shows a small cation leak conductance (Sonders *et al.* 1997), the more significant DAT-mediated conductance is thought to be mediated by Cl^- (Ingram *et al.* 2002).To explore if this conductance plays a role in the effect of the DAT on the short-term plasticity of DA release, $[Cl^-]_o$ has been modulated, and the effect on the short-term plasticity of DA release and its modulation by DAT inhibition explored.

Halving $[Cl^-]_o$ from 133 to 66.5 mM (replaced by gluconate to maintain osmolarity), reduced peak $[DA]_o$ across all stimulations (**Fig. 4.16 a,b**). The peak amplitude and duration of DA profiles across all stimulations was increased in cocaine, at both $[Cl^-]_o$ (**Fig. 4.16 a,b**). In both $[Cl^-]_o$, cocaine reduced the relationship between P2/P1 and IPI, decreasing P2/P1 at 10 ms IPI and increasing it at longer IPIs (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.05$; $n = 3$) (**Fig. 4.16 c,d**). Cocaine appeared to have similar effects at normal and low $[Cl^-]_o$ (**Fig. 4.16 c,d**). The effects of $[Cl^-]_o$ with and without cocaine were also examined. In control conditions, P2/P1 at 10 ms IPI was increased in low vs. control $[Cl^-]_o$ (Two-way ANOVA, *post hoc* Bonferroni t test, $p < 0.001$; $n = 3$) and there was a trend towards an increase at all other IPIs (**Fig. 4.16 e**). The effect of decreasing $[Cl^-]_o$ was similar in the presence of cocaine to in control conditions, with P2/P1 increased at all IPIs in low vs. normal $[Cl^-]_o$ (Two-way ANOVA, no significant interaction between $[Cl^-]_o$ and IPI but significant main effect of $[Cl^-]_o$, $p < 0.001$; $n = 3$) (**Fig. 4.16 f**). Hence, the effects of $[Cl^-]_o$ on P2/P1 do not appear to depend on DAT activity. To directly confirm that the effects of DAT inhibition on P2/P1 do not depend on $[Cl^-]_o$, the percentage change in P2/P1 with cocaine was compared at different $[Cl^-]_o$, and was not significantly different in control vs. low $[Cl^-]_o$ (Two-way ANOVA, $p > 0.05$; $n = 3$) (**Fig. 4.16 g**). A 3-way ANOVA also showed that there was no significant interaction between $[Cl^-]_o$, IPI and cocaine ($p < 0.05$; $n = 3$). Therefore, the effects of

the DAT on the short-term plasticity of DA release do not depend on $[Cl^-]_o$ and hence appear to be independent of the non-stoichiometric Cl^- conductance of the DAT.

$[Cl^-]_o$ is also involved in DA uptake itself, being co-transported with DA. Therefore it is possible that modulation of $[Cl^-]_o$ may also affect DA uptake. However, the rate of decay of DA profiles evoked by 1p was not significantly different in control and low $[Cl^-]_o$ (*$p>0.05$; $n = 3$, $R^2>0.84$*) (**Fig. 4.16 h**) suggesting that $[Cl^-]_o$ does not affect DA uptake.

4.3.11 DAT effects on the short-term plasticity of DA release are similar at physiological temperatures

In this thesis FCV experiments have been carried out at approximately 32^oC since acute slices are more viable at this temperature compared to higher, more physiological temperatures. However, since temperature was shown to significantly modulate the short-term plasticity of DA release (see **Fig. 4.9**), the effects of the DAT on the short-term plasticity of DA release have also been investigated at 37^oC. At 37^oC, cocaine increased the peak amplitude and duration of DA profiles across stimulations (**Fig. 4.17 a**) similarly to at 32^oC (compare with **Fig. 4.3 a**). Cocaine also modulated the short-term plasticity of release in a similar manner to at 32^oC, decreasing P2/P1 at 10 ms IPI and increasing it at 25-100 ms IPI (Two-way ANOVA, *post hoc* Bonferroni t test, *$p< 0.05$*; *$n = 3$*) (**Fig. 4.17 b** compare with **Fig. 4.3 c**). Therefore the role of the DAT in controlling the short-term plasticity of DA release, promoting the frequency sensitivity of release, is present at physiological temperatures.

4.4 Discussion

Following DA release in the striatum, DA is taken back up into DA terminals via the DAT, and repackaged and recycled for subsequent release. DA uptake via the DAT plays a key role in shaping extracellular striatal DA signals (Wightman and Zimmerman 1990; Jones *et al.* 1995;

Jones SR 1997) in an activity-dependent manner (Wightman and Zimmerman 1990; Garris *et al.* 1994; Garris and Rebec 2002). However, the DAT also shows ion channel-like conductances (Sonders *et al.* 1997; Ingram *et al.* 2002) and DAT inhibitors can increase DA release (Stamford *et al.* 1989; Lee *et al.* 2001; Robinson and Wightman 2004; Venton *et al.* 2006). These properties of the DAT suggest it may play a role in controlling DA release in addition to controlling uptake. In this chapter I have explored a role for the DAT in controlling the short-term plasticity of DA release. The results presented here suggest that the DAT promotes the frequency sensitivity of DA release, via multiple mechanisms at different frequencies of activity.

4.4.1 Cocaine modulates the frequency sensitivity of $[DA]_o$

The DAT inhibitor cocaine decreased the frequency sensitivity of $[DA]_o$, when nAChRs were inhibited. $[DA]_o$ is a compound measure of both DA release and DA uptake, so changes in either of these parameters may underlie the effect of cocaine on the frequency sensitivity of $[DA]_o$. The inhibition of uptake and hence the inhibition of DA removal from the extracellular space by cocaine likely contributes to the flattening of the frequency response (Garris *et al.* 1994; Garris and Rebec 2002). This is because, during release evoked by high frequency activity, there is little time for the DAT to remove DA from the extracellular space between release events, so extracellular DA signals summate. Therefore, DAT inhibition has little additional effect on extracellular DA summation during high frequency firing, as summation is already high. In contrast, during low frequency firing, there is time for the DAT to remove DA from the extracellular space between release events, so there is little extracellular summation of DA signals. DAT inhibition prevents this reuptake, greatly increasing extracellular summation of DA signals evoked by low frequency activity. Therefore, changes in extracellular summation likely partially underlie the relative increase in $[DA]_o$ evoked by low frequency stimulation observed with cocaine. However the effects of cocaine at high frequencies are harder to explain. In order

to investigate whether cocaine affects the plasticity of DA *release*, in addition to extracellular summation, the effect of cocaine on P2/P1 was explored.

4.4.2 Cocaine modulates the short-term plasticity of DA release

In contrast to $[DA]_o$, P2/P1 is a measure of the short-term plasticity of release that should be independent of DA uptake and summation. This is because uptake will approximately equally affect the amplitude of P2 and P1 (Cragg 2003). Cocaine significantly reduced the relationship between P2/P1 and IPI, in a similar manner to its modulation of the frequency sensitivity of $[DA]_o$. Therefore cocaine seems to alter the short-term plasticity of DA release itself in addition to its effects on extracellular summation. Cocaine had complex effects on the short-term plasticity of DA release, decreasing P2/P1 at short IPIs in both regions (decreasing facilitation) and, in dlCPu, increasing P2/P1 at longer IPIs (decreasing depression). In NAcC, cocaine had little effect on P2/P1 at longer IPIs. This may suggest that, in NAcC, the effects of cocaine on $[DA]_o$ at low frequencies may be largely due to changes in extracellular summation rather than release. The lack of effect of the DAT on the short-term plasticity of DA release at longer IPIs in NAcC may be due to the slightly higher P2/P1 at these longer IPIs in NAcC vs. dlCPu in control conditions (see **Fig. 3.2**), which is then not increased further by cocaine.

Cocaine is a fairly non-specific drug and is known to have a number of effects in addition to inhibiting the DAT. It was therefore explored whether the effect of cocaine on the short-term plasticity of DA release was specific to cocaine or due to a role of the DAT in controlling DA release.

4.4.3 The DAT modulates the short-term plasticity of DA release

All DAT inhibitors tested significantly reduced the frequency sensitivity of DA release, in a very similar manner to cocaine. Conversely the local anaesthetic, lidocaine, which is structurally related to cocaine but has little effect on DA uptake (Woodward *et al.* 1995, **Fig. 4.6**), had no

effect on the short-term plasticity of DA release. Therefore, the modulation of the short-term plasticity of DA release seen with cocaine appears to be due to its DAT inhibiting properties.

However these pharmacological experiments do not explore directly whether the endogenous activity of the DAT plays a role in the control of DA release. $DAT^{Cre/Cre}$ mice, which show a 40-50% decrease in striatal DAT protein levels (Bäckman *et al.* 2006), showed decreased DA uptake and a decreased frequency sensitivity of DA release. However, $DAT^{Cre/Cre}$ mice did not show the exact same relationship between P2/P1 and IPI as seen in cocaine. P2/P1 was reduced at short IPIs in $DAT^{Cre/Cre}$ compared to WT, but was also slightly decreased at longer IPIs in dlCPu, in contrast to the increase seen with DAT inhibition. This difference between $DAT^{Cre/Cre}$ animals and DAT inhibition may indicate that the effects of DAT inhibition at longer IPIs in dlCPu are due to a pharmacological effect of DAT inhibition rather than the blocking of an endogenous function of the DAT. Alternatively, it may reflect compensatory changes in DA handling in $DAT^{Cre/Cre}$ mice in response to chronic low uptake. Therefore, whilst overall these results support a role for endogenous DAT activity in controlling the short-term plasticity of DA release, there are differences between this model and acute pharmacological DAT inhibition. Therefore, acute models of decreased endogenous DAT activity were also explored.

Animals with dopaminergic lesions have a passive decrease in striatal DAT density and DA uptake, due to the loss of DAT-expressing DA terminal (Freed *et al.* 1995; Garris *et al.* 1997; Bergstrom and Garris 2003), and may also show a compensatory decrease in DAT activity in the remaining terminals (Bezard *et al.* 2000). Consistent with these previous reports, mice with unilateral 6-OHDA lesions showed decreased DA uptake in the hemisphere ipsilateral to the lesion. They also showed a decreased frequency sensitivity of DA release in the ipsilateral vs. contralateral hemisphere. This was similar to the effect of DAT inhibition on the short-term plasticity of DA release. There was no increase in P2/P1 at longer IPIs in lesioned animals, as seen in dlCPu with DAT inhibition. However, this may be because a different region in the CPu,

ventromedial CPu, had to be used in lesion experiments since this was where $[DA]_o$ was above the limit of detection, as described in 4.2.2. Additionally, although they are a more acute model of decreased DAT function than $DAT^{Cre/Cre}$ animals, 6-OHDA treated mice are also likely to show many compensatory changes in DA handling in addition to decreased DA uptake (Bergstrom *et al.* 2011). However, together with the above, these results also support a role for endogenous DAT activity in controlling the short-term plasticity of DA release.

Finally, in WT animals, decreasing experimental temperature to 25^{o}C was also found to mimic the effects of DAT inhibitors, reducing the frequency sensitivity of DA release, both decreasing P2/P1 at the shortest IPI and increasing it at slightly longer IPIs. Whilst decreasing temperature did decrease the rate of DA uptake, indicating decreased DAT activity, the effect was relatively small. Decreasing temperature will affect all enzymatic or stochastic processes in the DA terminal. Therefore, in addition to slowing DAT activity, decreasing temperature might mimic DAT inhibition by affecting processes downstream of the DAT. For example, the DAT has been shown to interact either physically or functionally with a number of presynaptic proteins via which it may alter transmitter release, including SNAP-25, PICK-1, syntaxin-1, α-syn, synaptogyrin-3 and synapsin (Lee *et al.* 2004; Torres 2006; Venton *et al.* 2006; Egana *et al.* 2009). These interactions may potentially be involved in the actions of DAT inhibitors on DA release plasticity, and will all be altered by decreasing temperature. Alternatively, presynaptic processes such as Ca^{2+}-dependent short-term facilitation will also be affected by temperature, and may underlie the effects of temperature on P2/P1.

Despite the limitations of each model, overall, these results using different DAT inhibitors and both chronic and acute models of decreased endogenous DAT function, support a role for endogenous DAT activity in the control of the short-term plasticity of DA release, promoting the frequency sensitivity of release.

4.4.4 Potential mechanisms underlying DAT control of the short-term plasticity of DA release:

4.4.4.1 DA transport

I then went on to investigate potential mechanisms underlying the effects of the DAT on the frequency sensitivity of DA release. Firstly, the effect of DA transport itself was explored. The short-term plasticity of DA release was investigated at E2, on a background of ongoing activity and DA release, and hence increased DA transport. This background of activity increased P2/P1 at all IPIs, but this effect appeared to be independent of the DAT. The DAT-mediated control of the short-term plasticity of DA release was unaffected by the background of activity, suggesting that the effect of the DAT on the short-term plasticity of DA release is independent of the level of actual DA translocation.

4.4.4.2 DAT effects at short and long IPIs are dissociable

The DAT-mediated control of the short-term plasticity of DA release appears complex, with different effects at different timescales, particularly in dlCPu. DAT inhibition decreases P2/P1 at short IPIs, but increases it at longer IPIs in dlCPu. Using different concentrations of cocaine, it was found that these two effects were dissociable. In dlCPu, low concentrations of cocaine increased P2/P1 at longer IPIs, but had no effect at shorter IPIs. This is therefore consistent with the DAT having two distinct effects on the short-term plasticity of DA release over different timescales, rather than one complex effect.

The fact that cocaine has two dissociable effects on P2/P1 may suggest that there are two different cocaine binding sites involved in these actions. These could reflect two different cocaine targets. However, this seems unlikely since other DAT inhibitors have similar effects on the short-term plasticity of DA release, strongly implicating the DAT in the effects of cocaine. Then again they could reflect two different cocaine binding sites on the DAT. This is possible; the site of the cocaine binding site on the DAT is still controversial, and multiple sites have been

suggested (Beuming *et al.* 2008; Huang *et al.* 2009). Alternatively, the fact that these two effects of DAT inhibition can be dissociated may be due to them having different thresholds of DAT inhibition or being dependent on different outcomes of DAT inhibition, as opposed to different cocaine binding sites. Indeed, the low concentration of cocaine slowed DA uptake but did not significantly increase DA release. This hints that distinct effects of DAT inhibition may underlie these two distinct effects on the plasticity of release at different timescales.

4.4.4.3 Synapsins

The above experiments using a low concentration of cocaine hinted that the increase in $[DA]_o$ with DAT inhibition might be necessary for the decrease in P2/P1 at short IPIs. This would be consistent with the classic inverse relationship between initial and subsequent release, discussed in chapter 3. The mechanism by which cocaine can increase DA release has been shown to be via the mobilisation of a synapsin-dependent reserve pool of DA vesicles, since in triple synapsin knockout mice cocaine has little effect on peak $[DA]_o$ (Venton *et al.* 2006; Kile *et al.* 2010). Synapsins are a family of presynaptic phosphoproteins thought to play important roles in maintaining vesicle pools (Li *et al.* 1995; Gitler *et al.* 2004). The synapsin III isoform is particularly implicated in the control of the reserve pool in DA neurons, and its modulation by cocaine (Kile *et al.* 2010). This is because only this isoform appears to be expressed in these cells (Pieribone *et al.* 2002; Bogen *et al.* 2006), and DA release is similarly increased in S3KO and triple knockout animals (Kile *et al.* 2010). Therefore the effects of DAT inhibition on the short-term plasticity of DA release were explored in S3KO mice. In drug-free conditions, these animals showed enhanced DA release compared to WTs, although the size of this increase was less than had been reported previously (Kile *et al.* 2010). Nevertheless, these results support a role for synapsin III in controlling striatal DA release. However, cocaine still greatly increased peak $[DA]_o$ in these animals similarly to in WTs. Cocaine also altered the short-term plasticity of DA release in S3KO mice similarly to in WTs. Thus, the effects of DAT inhibition on DA release are independent of synapsin III. Since in triple synapsin knockout mice, cocaine has no effect on DA release (Venton

et al. 2006; Kile *et al.* 2010), but here cocaine similarly affected DA release in S3KOs and WTs, other synapsin isoforms must be functional in DA terminals. Indeed whilst several reports argue against the expression of other synapsin isoforms in DA neurons (Bogen *et al.* 2006), there is a reported interaction between synapsin I and DAT in brain homogenates (Maiya *et al.* 2007). Since the DAT is only thought to be expressed in DA neurons (Freed *et al.* 1995), this implies that synapsin I must be expressed in DA neurons. It appears then that different synapsin isoforms play different roles in controlling vesicle pools in DA terminals. Synapsin III controls basal DA release, since peak $[DA]_o$ is increased in these animals, so is perhaps involved in the control of the recycling pool of DA vesicles. However other isoforms appear to control the reserve pool of DA vesicles and mediate the cocaine-induced mobilisation of these vesicles.

4.4.4.4 Calcium

To further probe a role for the synapsin-dependent mechanism in the control of the short-term plasticity of DA release, the effect of $[Ca^{2+}]_o$ was explored, since the synapsin-dependent change in DA release has been reported to be greater in low $[Ca^{2+}]_o$ (Kile *et al.* 2010). Decreasing $[Ca^{2+}]_o$ greatly increased the effect of DAT inhibition on the short-term plasticity of DA release at longer, but not short, IPIs. In addition to its effects on the short-term plasticity of DA release, $[Ca^{2+}]_o$ significantly alters P_r. However, decreasing stimulation current, which decreased peak $[DA]_o$ evoked by 1p similarly to halving $[Ca^{2+}]_o$, had no effect on the DAT-mediated control of the short-term plasticity of DA release. This suggests that the effects of $[Ca^{2+}]_o$ on the DAT control of the short-term plasticity of release are specific to Ca^{2+} and not simply a result of changes in P_r.

It is therefore possible that the Ca^{2+}- and synapsin-dependent mechanism by which endogenous DAT activity appears to limit initial DA release, further limits subsequent DA release over 25-200 ms IPIs, and underlies the effect of the DAT on the short-term plasticity of DA release at longer IPIs.

4.4.4.5 DAT Cl^- conductance

It was then explored whether the non-transport associated Cl^- conductance of the DAT (Ingram *et al.* 2002) might play a role in the control of the short-term plasticity of DA release. Decreasing $[Cl^-]_o$ increased P2/P1, but this effect appeared to be independent of the DAT. Decreasing $[Cl^-]_o$ did not alter the effect of cocaine on the short-term plasticity of release, suggesting that the DAT-mediated Cl^- conductance does not play role in the DAT-mediated control of the short-term plasticity of DA release.

4.4.4.6 Summary of potential mechanisms

The above results suggest that the effects of the DAT on the short-term plasticity of DA release do not depend on either DA translocation via the DAT or on the DAT-mediated Cl^- conductance. The opposing effects of the DAT on the short-term plasticity of release at short and longer IPIs in dlCPu are dissociable, suggesting that they occur via distinct mechanisms or have different thresholds. The effect of the DAT on the short-term plasticity of release at longer IPIs is greater at low $[Ca^{2+}]_o$, hinting that this effect of the DAT may be mediated by the synapsin-dependent effect of the DAT on a reserve pool of DA vesicles (Venton *et al.* 2006; Kile *et al.* 2010), although this is not dependent on the synapsin III isoform. The mechanism underlying the DAT control of P2/P1 at very short IPIs has not been determined, although results with a low concentration of cocaine hinted that it may depend on the DAT control of the amplitude of DA release. It is possible that the increase in initial release seen with DAT inhibition may increase release-dependent depression of release, decreasing P2/P1 at short IPIs as discussed in chapter 3. However, as discussed, the increase in initial release seen with DAT inhibition has been reported to be dependent on $[Ca^{2+}]_o$ (Kile *et al.* 2010) but the effect of the DAT on P2/P1 at short IPIs was not found to vary with $[Ca^{2+}]_o$. This may be due to different thresholds of the two effects: that decreasing $[Ca^{2+}]_o$ increases the synapsin-dependent mobilisation of reserve vesicles sufficiently to alter the effect of cocaine on the short-term plasticity of DA release at longer IPIs but not sufficiently to alter P2/P1 at short IPIs. Alternatively, it may indicate that the effect of the DAT on

the short-term plasticity of DA release at very short IPIs occurs via another, unidentified mechanism, potentially involving other protein-protein interactions of the DAT, such as with SNAP-25 or syntaxin-1 (Lee *et al.* 2004; Torres 2006).

4.4.5 Behavioural relevance and implications

Finally in this chapter it was shown that DAT inhibition flattens the frequency response of DA release at a physiological temperature, similarly to at the lower standard experimental temperature, confirming that this may be a physiologically relevant effect. This alteration of the frequency sensitivity of DA release with DAT inhibition, when nAChRs are not active, may have great behavioural relevance. As discussed previously, ChIs show pauses in firing in response to reward-related events (Aosaki *et al.* 1995; Morris *et al.* 2004; Apicella *et al.* 2009). These pauses are thought to lead to decreased ACh actions at nAChRs (Cragg 2006). Coincident with these pauses, DA neurons switch from low frequency to high frequency bursts of activity, in response to behaviourally significant events (Schultz 2007). This switch in firing pattern, when nAChRs are not active, is thought to cause a phasic increase in DA release in the striatum (Chergui *et al.* 1994; Garris *et al.* 1994; Richardson and Gratton 1996; Venton *et al.* 2003), which encodes important reward-related information. The results presented in this chapter suggest a previously unidentified role for the DAT in maintaining this filtering of different DA neuron firing patterns into large changes in striatal DA release. When nAChRs are inhibited, the DAT appears to promote the contrast between DA signals evoked by high frequency reward-related firing and low frequency tonic firing, which may be hypothesised to enhance the transmission of reward-related information to postsynaptic neurons. This suggests that DAT inhibitors, including therapeutic drugs such as methylphenidate, and drugs of abuse such as cocaine, will alter how DA terminals filter firing patterns into striatal DA release, flattening the frequency sensitivity of release, in addition to simply increasing and prolonging striatal DA signals. Together with the increased extracellular summation of dopamine seen with DAT inhibition, the reduced frequency

sensitivity of DA release may be hypothesised to decrease the contrast between reward-related and tonic striatal DA signals. This will likely impair detection of reward-related signals by DA target neurons, and may have downstream behavioural effects and contribute to the impairments in reward-related decision making seen with DAT inhibitors (Grant *et al.* 2000; Simon *et al.* 2007; Porter *et al.* 2011).

The DAT appears to have slightly different effects on the short-term plasticity of DA release in dlCPu and NAcC. In dlCPu, DAT inhibition decreases facilitation of DA release only at the shortest IPIs (10 ms), which are rarely reported for DA neurons *in vivo* (Grace and Bunney 1984; Hyland *et al.* 2002), but also decreases depression of DA release at longer IPIs. In contrast, in the NAcC, cocaine decreases subsequent DA release at IPIs below 40 ms, which are within the representative range of DA neuron firing *in vivo* (Kiyatkin and Rebec 1998; Hyland *et al.* 2002), but has no effect on subsequent release at longer IPIs. Therefore, the precise effects of DAT inhibition on DA signals *in vivo* will depend on a complex interplay of firing frequency and region, which may contribute to the reported different effects of DAT inhibitors on DA signalling in the two striatal regions (Carboni *et al.* 1989; Cass *et al.* 1992; Kuczenski and Segal 1992; Wu *et al.* 2001).

4.5 Summary

In this chapter a previously unidentified role for the DAT in controlling the short-term plasticity of DA release had been demonstrated. This occurs via multiple mechanisms, with different time scales. Over very short interpulse intervals DAT inhibition inhibits facilitation of DA release. The mechanism underlying this effect is uncertain but may be due to increases in initial DA release leading to decreased subsequent release. Over longer interpulse intervals, inhibition of the DAT decreases depression of DA release in dlCPu, in a Ca^{2+}-dependent manner. These effects on the short-term plasticity of release reveal an intriguing and previously unexplored role for a

neurotransmitter uptake transporter, and may have implications for how DAT inhibitor drugs alter the contrast between behaviourally significant DA signals.

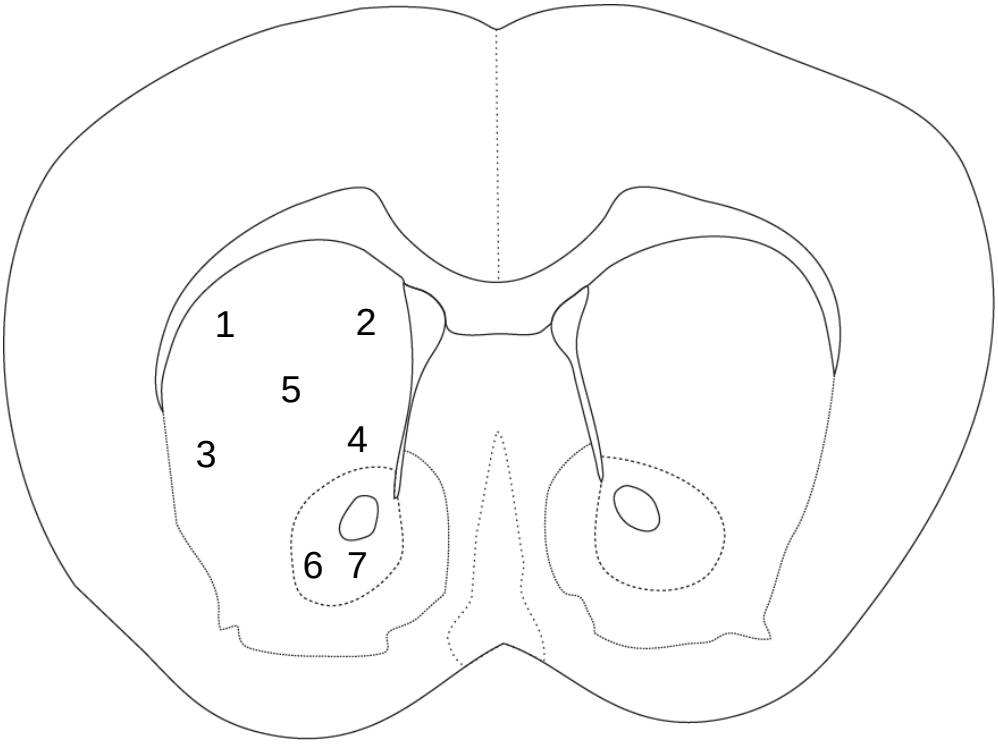

Figure 4.1. Diagram of coronal brain slice showing locations of recording sites used in experiments comparing $[DA]_o$ in S3KO vs. WT mice. To obtain representative measurements of striatal DA release, $[DA]_o$ was monitored at 5 sites in CPu: 1 - dorsolateral, 2 - dorsomedial, 3 - ventrolateral, 4 - ventromedial, 5 - central, and 2 sites in NAcC: 6 - lateral, 7 - medial.

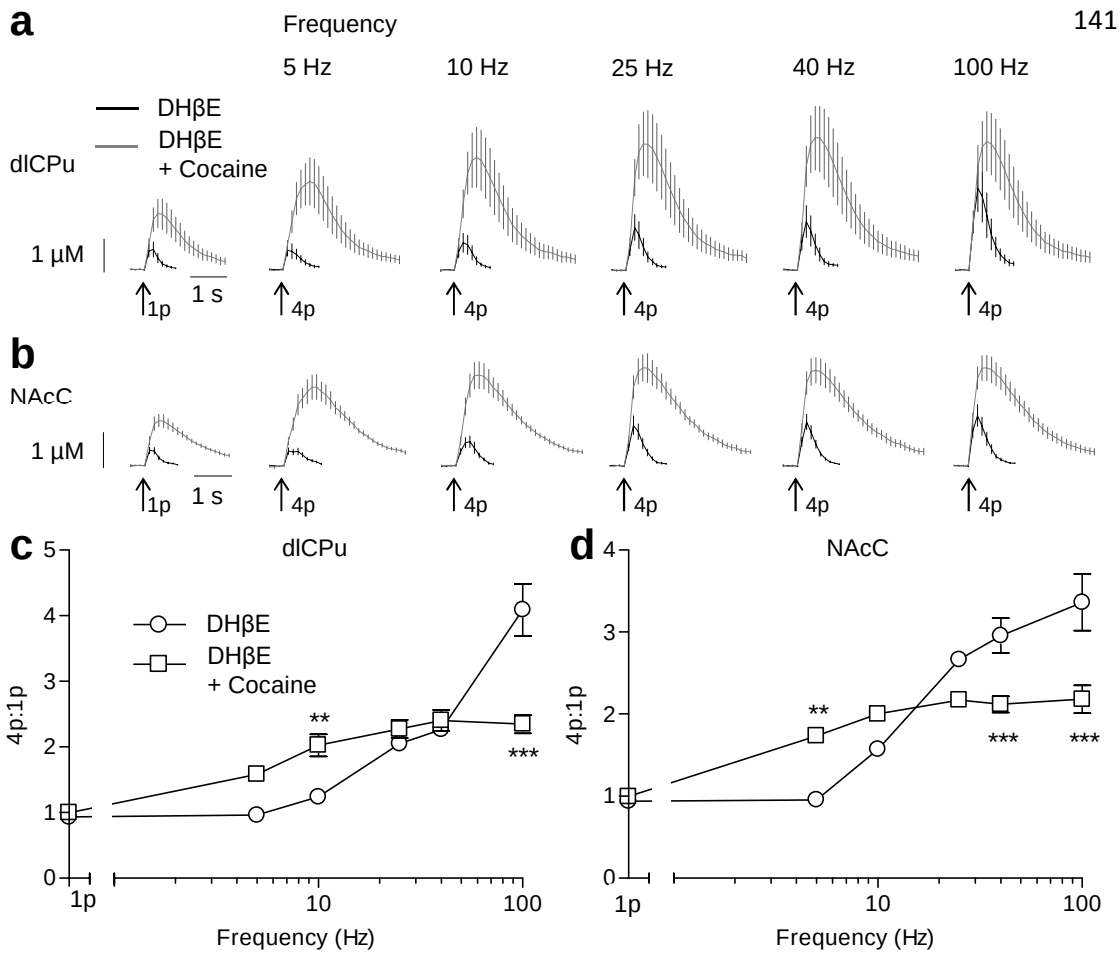

Figure 4.2. DAT inhibition using cocaine alters the frequency sensitivity of $[DA]_o$. (a,b) Profiles of mean $[DA]_o$±SEM against time after single pulse or four pulse train stimulation (arrow)(1p or 4p, 5-100 Hz) with nAChRs inhibited (DHβE, 1 µM) before and after cocaine (5 µM), in dlCPu (a) and NAcC (b). **(c,d)** Mean ratio ±SEM of peak $[DA]_o$ evoked by train stimulation to mean peak $[DA]_o$ evoked by 1p (4p:1p), against stimulation frequency, with DHβE (1 µM), before and after cocaine (5 µM), in dlCPu (c), and NAcC (d). In dlCPu, cocaine significantly reduced 4p:1p at 100 Hz but increased it at 10 Hz (Two-way ANOVA, Main effect of cocaine: $p > 0.05$ $F_{1,24}$=0.03, Main effect of frequency: $p < 0.001$ $F_{5,24}$=54.11, Interaction: $p < 0.001$, $F_{5,24}$=16.72, *post hoc* Bonferroni t test, $p < 0.01$ at 10 and 100 Hz; $n = 3$). In NAcC, cocaine significantly reduced 4p:1p at 40-100 Hz but increased it at 5 Hz (Two-way ANOVA, Main effect of cocaine: $p > 0.05$ $F_{3,240}$=2.00, Main effect of frequency: $p < 0.001$ $F_{2,240}$=272.99, Interaction: $p < 0.001$, $F_{15,240}$=28.79, *post hoc* Bonferroni t test, $p < 0.01$ at 5, 40 and 100 Hz; $n = 3$).

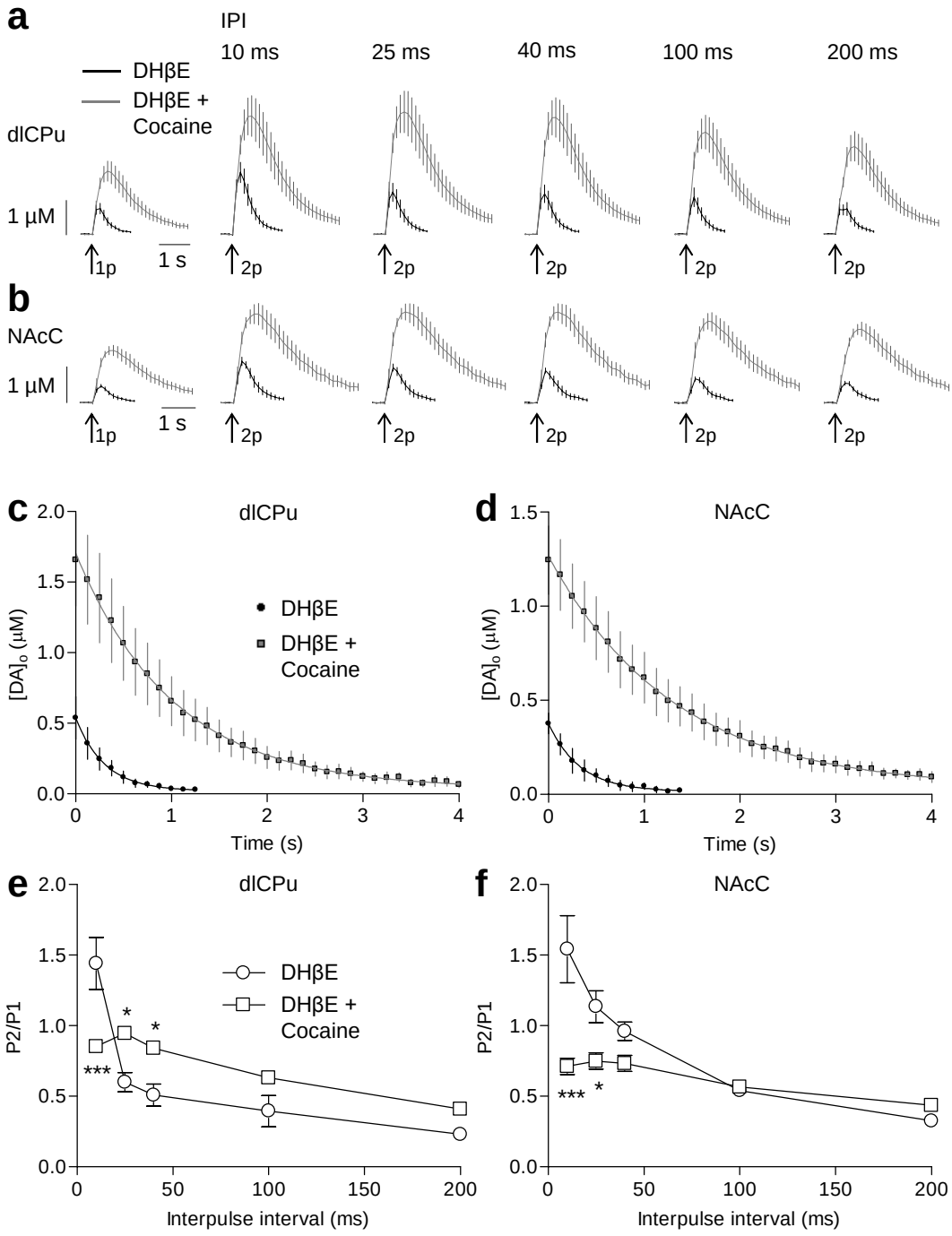

Figure 4.3. DAT inhibition using cocaine alters short-term plasticity of DA release. (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), with nAChRs inhibited (DHβE, 1 µM) before and after cocaine (5 µM), in dlCPu (a) and NAcC (b). **(c,d)** Decay phases of profiles of $[DA]_o$±SEM evoked by 1p, against time after start of decay phase, in dlCPu (c) and NAcC (d), with DHβE (1 µM), before and after cocaine (5 µM). Curve fits are one phase exponential decays, $R^2>0.96$. In both regions rate constants of decay are significantly different between drug conditions (dlCPu: K=3.28 s^{-1} control, K=0.97 s^{-1} cocaine, $p < 0.0001$, $F_{1,42}$=119.3*; n = 4;* NAcC: F K=3.04 s^{-1} control, K=0.76 s^{-1} cocaine, $p < 0.0001$, $F_{1,41}$=226.4*; n = 4*). **(e,f)** Mean P2/P1±SEM against IPI in dlCPu (e) and NAcC (f), before and after cocaine (5 µM). In dlCPu, cocaine significantly decreased P2/P1 at 10 ms IPI, but increased in at 25-40 ms IPI (Two-way ANOVA, Main effect of cocaine: $p > 0.05$ $F_{1,30}$=3.92, Main effect of IPI: $p < 0.001$ $F_{4,30}$=30.08, Interaction: $p < 0.001$, $F_{4,30}$=11.97, *post hoc* Bonferroni t test, $p < 0.05$ at 10-40 ms IPI; $n = 4$). In NAcC, cocaine significantly decreased P2/P1 at 10-25 ms IPI (Two-way ANOVA, Main effect of cocaine: $p < 0.001$ $F_{1,30}$=19.36, Main effect of IPI: $p < 0.001$ $F_{4,30}$=20.36, Interaction: $p < 0.001$, $F_{4,30}$=7.88, *post hoc* Bonferroni t test, $p < 0.05$ at 10-25 ms IPI; $n = 4$).

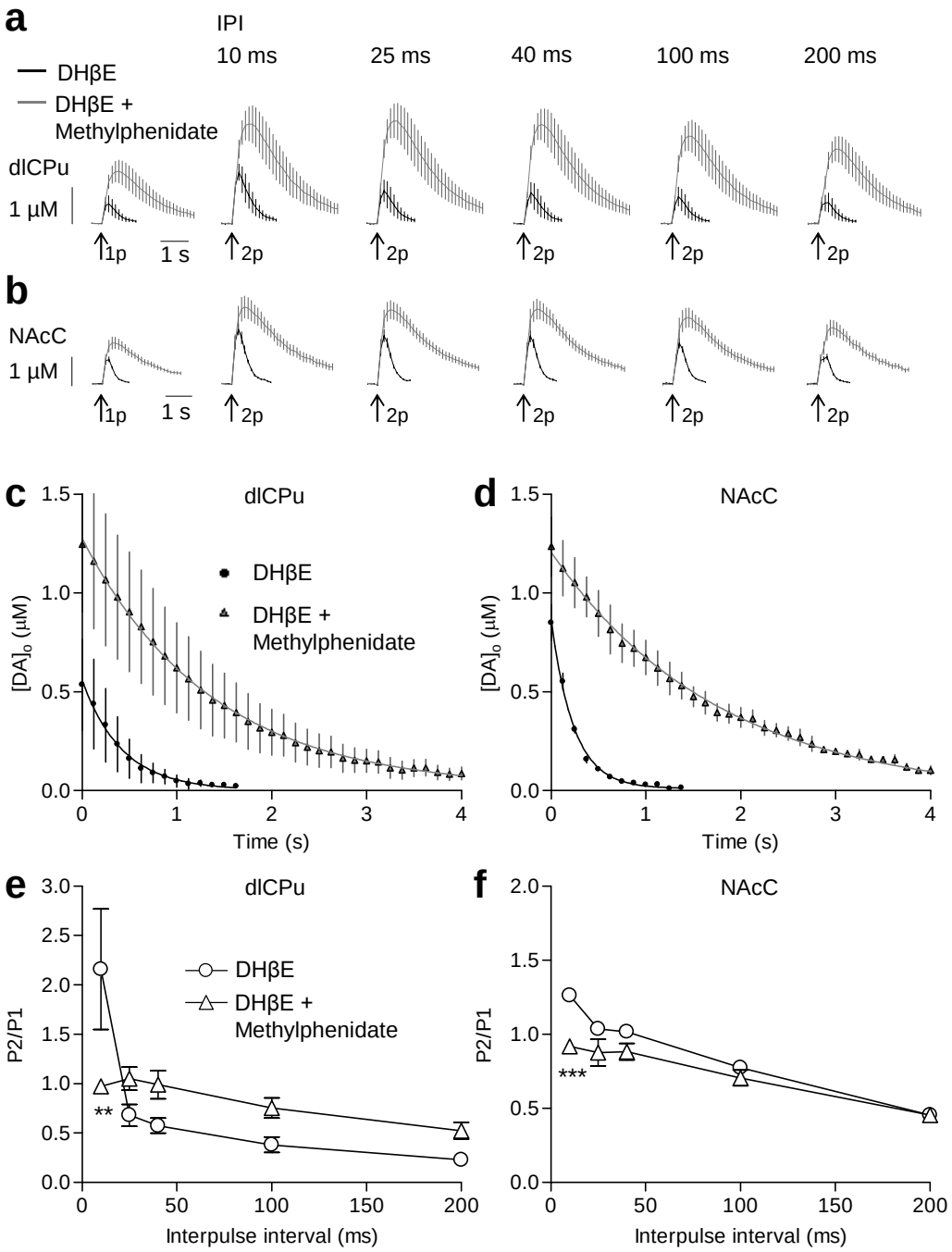

Figure 4.4. DAT inhibition using methylphenidate has a similar effect on the short-term plasticity of DA release to cocaine. (a,b) Profiles of mean $[DA]_o$±SEM against time, after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), with nAChRs inhibited (DHβE, 1 µM) before and after methylphenidate (5 µM), in dlCPu (a) and NAcC (b). **(c,d)** Decay phases of profiles of $[DA]_o$±SEM evoked by 1p, against time after start of decay phase in dlCPu (c) and NAcC (d), with DHβE (1 µM), before and after methylphenidate (5 µM). Curve fits are one phase exponential decays, $R^2>0.92$. In both regions rate constants of decay are significantly different between drug conditions (dlCPu: K=2.75 s^{-1} control, K=0.73 s^{-1} methylphenidate, $p<0.0001$, $F_{1,53}$=263.4*; n=5;* NAcC: K=4.20 s^{-1} control, K=0.57 s^{-1} methylphenidate, $p<0.0001$, $F_{1,55}$=812.2*; n=3*). **(e,f)** Mean P2/P1±SEM against IPI in dlCPu (e) and NAcC (f), with DHβE (1 µM), before and after methylphenidate (5 µM).Methylphenidate significantly decreased P2/P1 at 10 ms IPI in both regions (Two-way ANOVA, dlCPu: Main effect of methylphenidate: $p > 0.05$ $F_{1,40}$=0.16, Main effect of IPI: $p < 0.001$ $F_{4,40}$=9.05, Interaction: $p < 0.01$, $F_{4,40}$=5.32, *post hoc* Bonferroni t test, $p < 0.01$ at 10 ms IPI; NAcC: Main effect of methylphenidate: $p < 0.001$ $F_{1,20}$=23.89, Main effect of IPI: $p < 0.001$ $F_{4,20}$=58.98, Interaction: $p < 0.05$, $F_{4,20}$=3.99, *post hoc* Bonferroni t test, $p < 0.001$ at 10 ms IPI; $n = 3$-5) but in dlCPu there was a trend towards increased P2/P1 at longer IPIs..

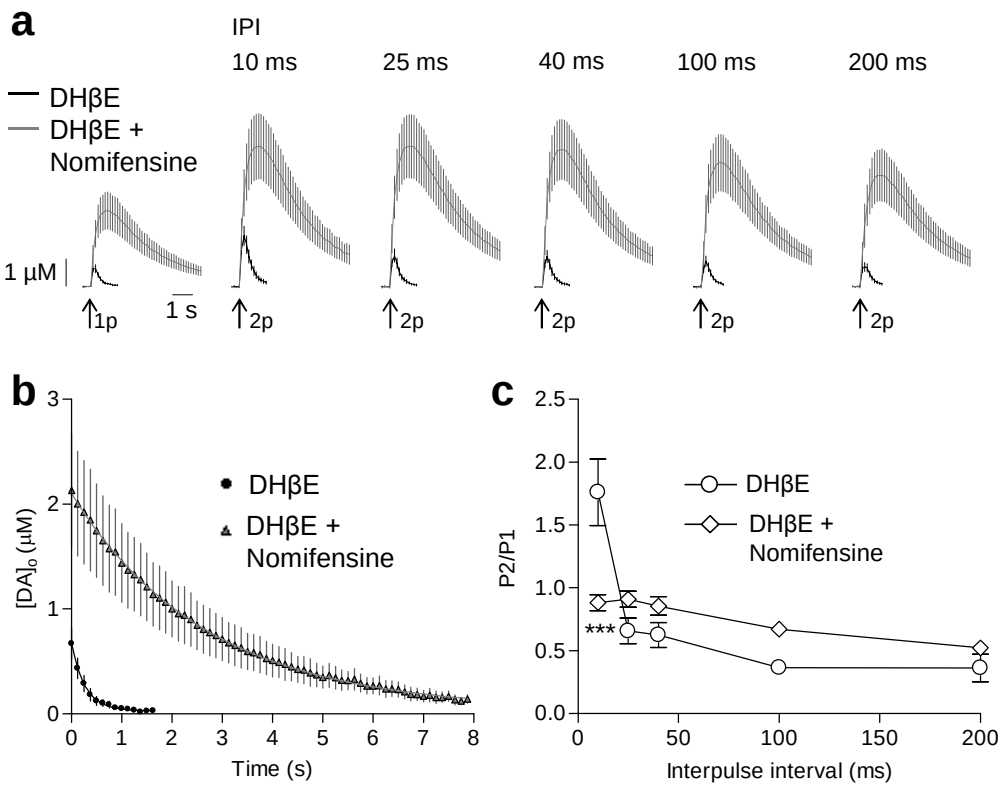

Figure 4.5. DAT inhibition using nomifensine has a similar effect on the short-term plasticity of DA release to cocaine. (a) Profiles of mean $[DA]_o$±SEM against time, after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms) with nAChRs inhibited (DHβE, 1 µM), before and after nomifensine (10 µM), in dlCPu. **(b)** Decay phases of profiles of $[DA]_o$±SEM evoked by 1p, against time after start of decay phase, with DHβE (1 µM), before and after nomifensine (10 µM). Curve fits are one phase exponential decays, $R^2>0.99$. Rate constant of decay is significantly different between drug conditions (K=3.65 s^{-1} control, K=0.37 s^{-1} nomifensine, $p < 0.0001$, $F_{1,72}$=667.9*; n = 5*). **(c)** Mean P2/P1±SEM against IPI, with DHβE (1 µM), before and after nomifensine (10 µM). Nomifensine significantly decreased P2/P1 at 10 ms IPI (Two-way ANOVA, Main effect of nomifensine: $p > 0.05$ $F_{1,40}$=0.04, Main effect of IPI: $p < 0.001$ $F_{4,40}$=19.46, Interaction: $p < 0.001$, $F_{4,40}$=10.31, *post hoc* Bonferroni t test, $p < 0.01$ at 10 ms IPI; $n = 5$) but caused a trend towards an increase in P2/P1 at longer IPIs.

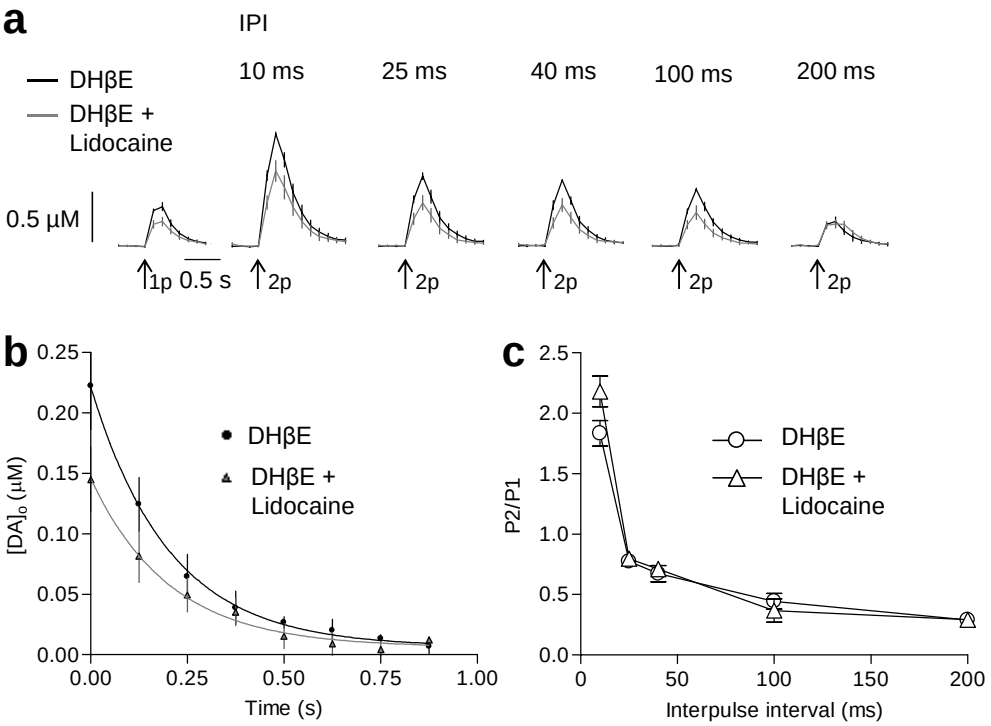

Figure 4.6. VGSC inhibition using lidocaine does not have a similar effect on short-term plasticity of DA release to cocaine. (a) Profiles of mean $[DA]_o$±SEM against time, after single or paired pulse stimulations (arrow) (1p or 2p, IPI 10-200 ms), with nAChRs inhibited (DHβE, 1 µM), before and after lidocaine (5 µM), in dlCPu. **(b)** Decay phases of profiles of $[DA]_o$±SEM evoked by 1p, against time after start of decay phase, with DHβE (1 µM), before and after lidocaine (5 µM). Curve fits are one phase exponential decays, $R^2>0.87$. Rate constant of decay is not significantly different between drug conditions ($p > 0.05$ $F_{1,10}$=1.32*; n = 3*). **(c)** Mean P2/P1±SEM against IPI, with DHβE (1 µM), before and after lidocaine (5 µM). Lidocaine had no significant effect on P2/P1 (Two-way ANOVA, Main effect of lidocaine: $p > 0.05$ $F_{1,80}$=1.92, Main effect of IPI: $p < 0.001$ $F_{4,80}$=163.58, Interaction: $p > 0.05$, $F_{4,80}$=2.29, $n = 3$). Data collected by Min-Yee Tseu (MBiochem).

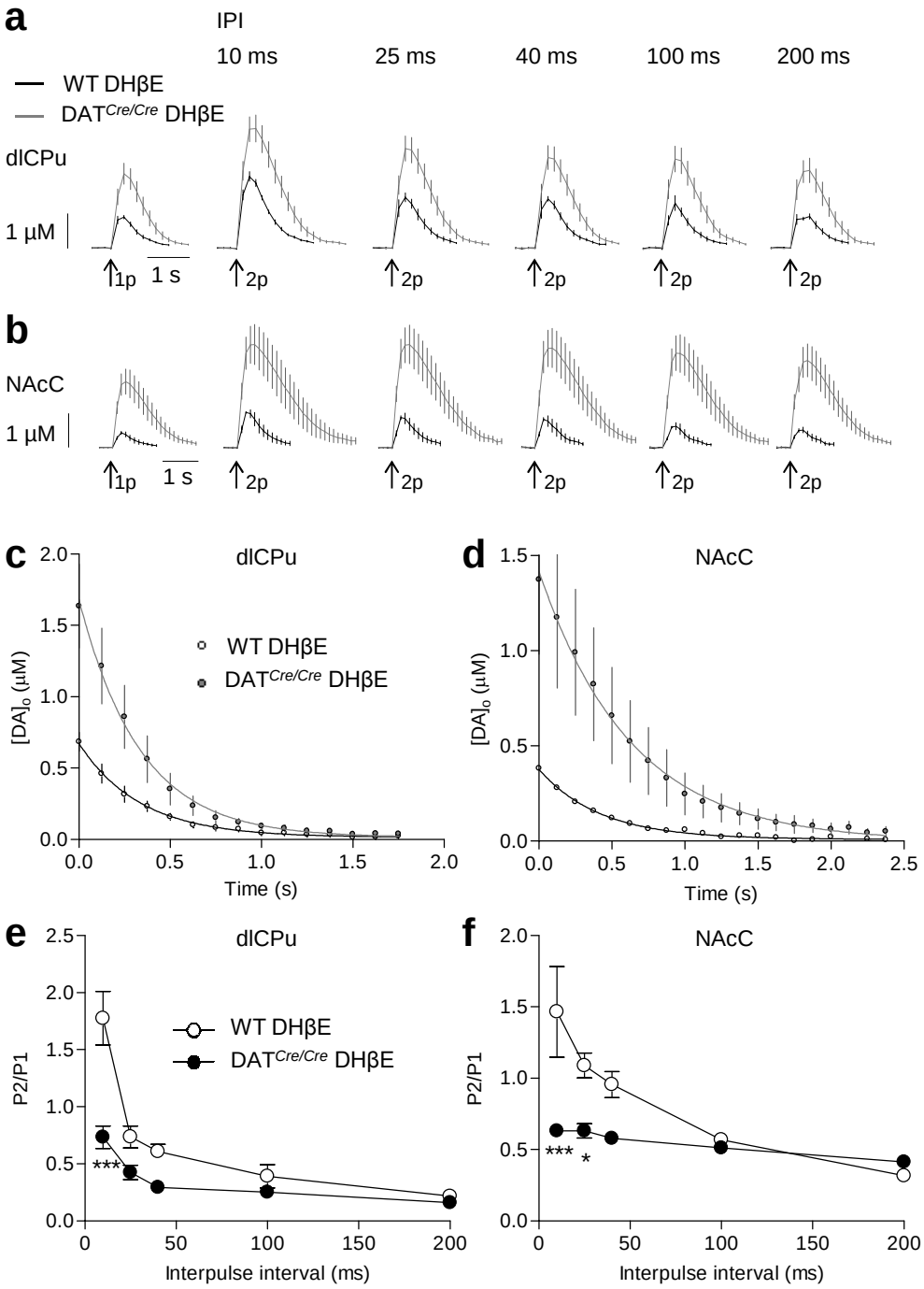

Figure 4.7. DAT$^{Cre/Cre}$ mice, with decreased DAT expression, mimic the effect of DAT inhibition on DA release plasticity. (a,b) Profiles of mean $[DA]_o$±SEM against time, after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), with nAChRs inhibited (DHβE, 1 µM), in WT and DAT$^{Cre/Cre}$ mice, in dlCPu (a) and NAcC (b). **(c,d)** Decay phases of profiles of $[DA]_o$±SEM evoked by 1p, against time after start of decay phase in dlCPu (c) and NAcC (d), with DHβE (1 µM), in WT and DAT$^{Cre/Cre}$ mice. Curve fits are one phase exponential decays, $R^2>0.98$. In dlCPu rate constant of decay is not significantly different between genotypes ($p > 0.05$, $F_{1,19}$=0.79; $n = 3$). In NAcC, rate constant of decay is significantly slower in DAT$^{Cre/Cre}$ vs. WT (K=2.39 s^{-1} in WT, K=1.59 s^{-1} in DAT$^{Cre/Cre}$, $p < 0.0001$, $F_{1,26}$=20.00; $n = 3$). **(e,f)** Mean P2/P1±SEM against IPI in dlCPu (e) and NAcC (f), with DHβE (1 µM), in WT and DAT$^{Cre/Cre}$. In dlCPu, P2/P1 was significantly lower in DAT$^{Cre/Cre}$ compared to WT at 10 ms IPI (Two-way ANOVA, Main effect of genotype: $p < 0.001$, $F_{1,35}$=19.33, Main effect of IPI: $p < 0.001$ $F_{4,35}$=19.10, Interaction: $p < 0.01$, $F_{4,35}$=4.21, *post hoc* Bonferroni t test, $p < 0.01$ at 10 ms IPI; $n = 3$). In NAcC, P2/P1 was significantly lower in DAT$^{Cre/Cre}$ compared to WT at 10 and 25 ms IPI (Two-way ANOVA, Main effect of genotype: $p < 0.001$, $F_{1,20}$=21.45, Main effect of IPI: $p < 0.001$ $F_{4,20}$=11.67, Interaction: $p < 0.01$, $F_{4,20}$=5.35, *post hoc* Bonferroni t test, $p < 0.05$ at 10-25 ms IPI; $n = 3$).

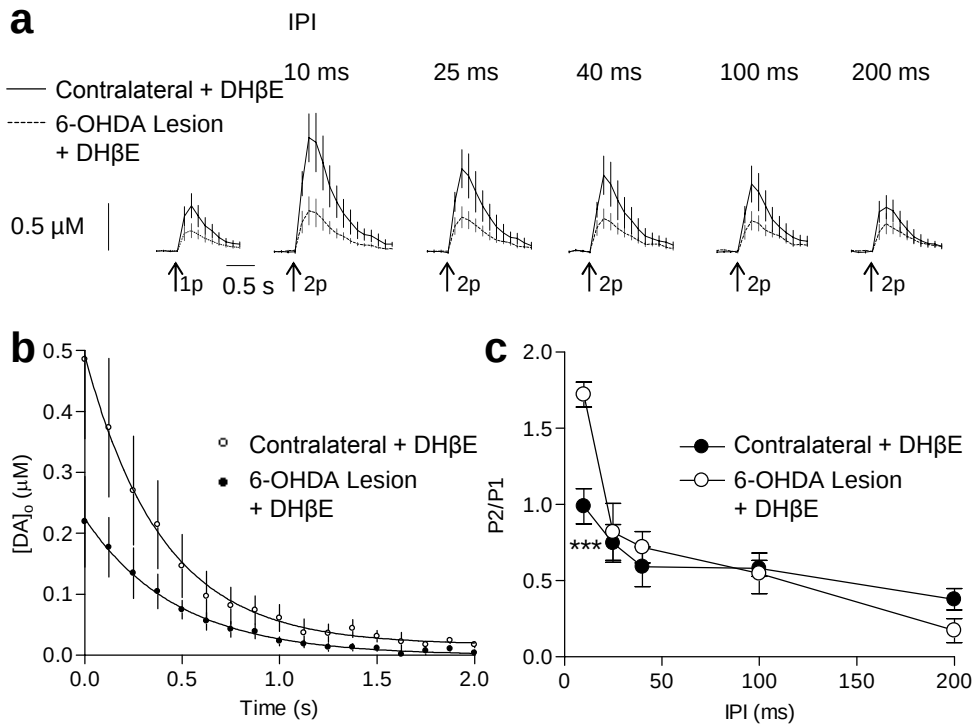

Figure 4.8. 6-OHDA lesions decrease the rate of DA uptake and mimic the effect of DAT inhibition on DA release plasticity. (a) Profiles of mean $[DA]_o$±SEM against time, after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), with nAChRs inhibited (DHβE, 1 µM), in contralateral and ipsilateral hemispheres to 6-OHDA lesion. **(b)** Decay phases of profiles of $[DA]_o$±SEM evoked by 1p, against time after start of decay phase, with DHβE (1 µM), in contralateral and ipsilateral hemispheres. Curve fits are one phase exponential decays, $R^2>0.98$. Rate constant of decay is significantly different between hemispheres (K=2.51 s^{-1} in contralateral hemisphere, K=2.12 s^{-1} in ipsilateral hemisphere, $p < 0.01$, $F_{1,28}$=8.68; $n = 3$). **(c)** Mean P2/P1±SEM against IPI, with DHβE (1 µM), in contralateral and ipsilateral hemispheres. P2/P1 was significantly reduced at 10 ms IPI in the ipsilateral compared to the contralateral hemisphere (Two-way ANOVA, Main effect of lesion: $p > 0.05$, $F_{1,25}$=3.77, Main effect of IPI: $p < 0.001$ $F_{4,25}$=24.70, Interaction: $p < 0.01$, $F_{4,25}$=4.91, *post hoc* Bonferroni t test, $p < 0.001$ at 10 ms IPI; $n = 3$).

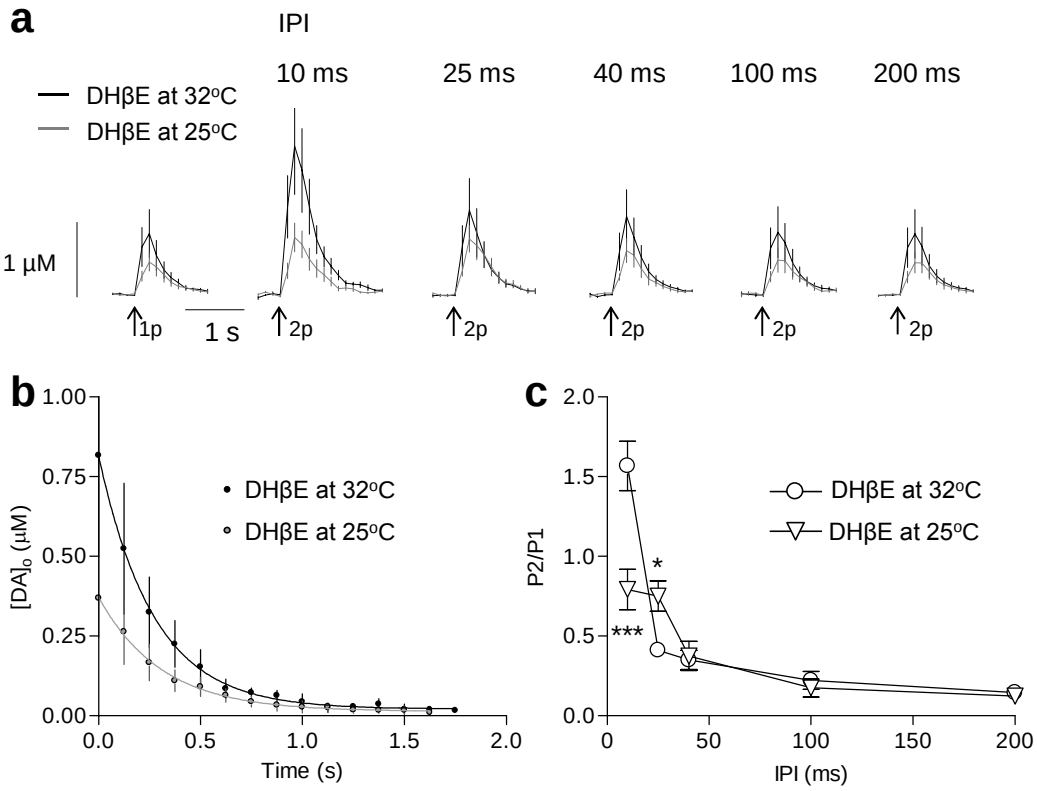

Figure 4.9. Decreasing temperature decreases the rate of DA uptake and mimics the effect of DAT inhibition on DA release plasticity. (a) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow) (1p or 2p, IPI 10-200 ms), with nAChRs inhibited (DHβE, 1 µM), at 32 or 25°C. **(b)** Decay phases of profiles of $[DA]_o$±SEM evoked by 1p, against time after start of decay phase, with DHβE (1 µM), at 32 and 25°C. Curve fits are one phase exponential decays, $R^2>0.95$. Rate constant of decay is significantly different between temperatures (K=3.71 s^{-1} 32°C, K=3.25 s^{-1} 25°C, $p < 0.05$, $F_{1,19}$=5.94; $n = 3$). **(c)** Mean P2/P1±SEM against IPI in dlCPu, with DHβE (1 µM), at 32 and 25°C. P2/P1 was decreased at 10 ms IPI but increased P2/P1 at 25 ms IPI at 25°C vs. 32°C (Two-way ANOVA, Main effect of temperature: $p > 0.05$, $F_{1,20}$=3.34, Main effect of IPI: $p < 0.001$ $F_{4,20}$=51.90, Interaction: $p < 0.001$, $F_{4,20}$=12.26, *post hoc* Bonferroni t test, $p < 0.05$ at 10-25 ms IPI; $n = 3$).

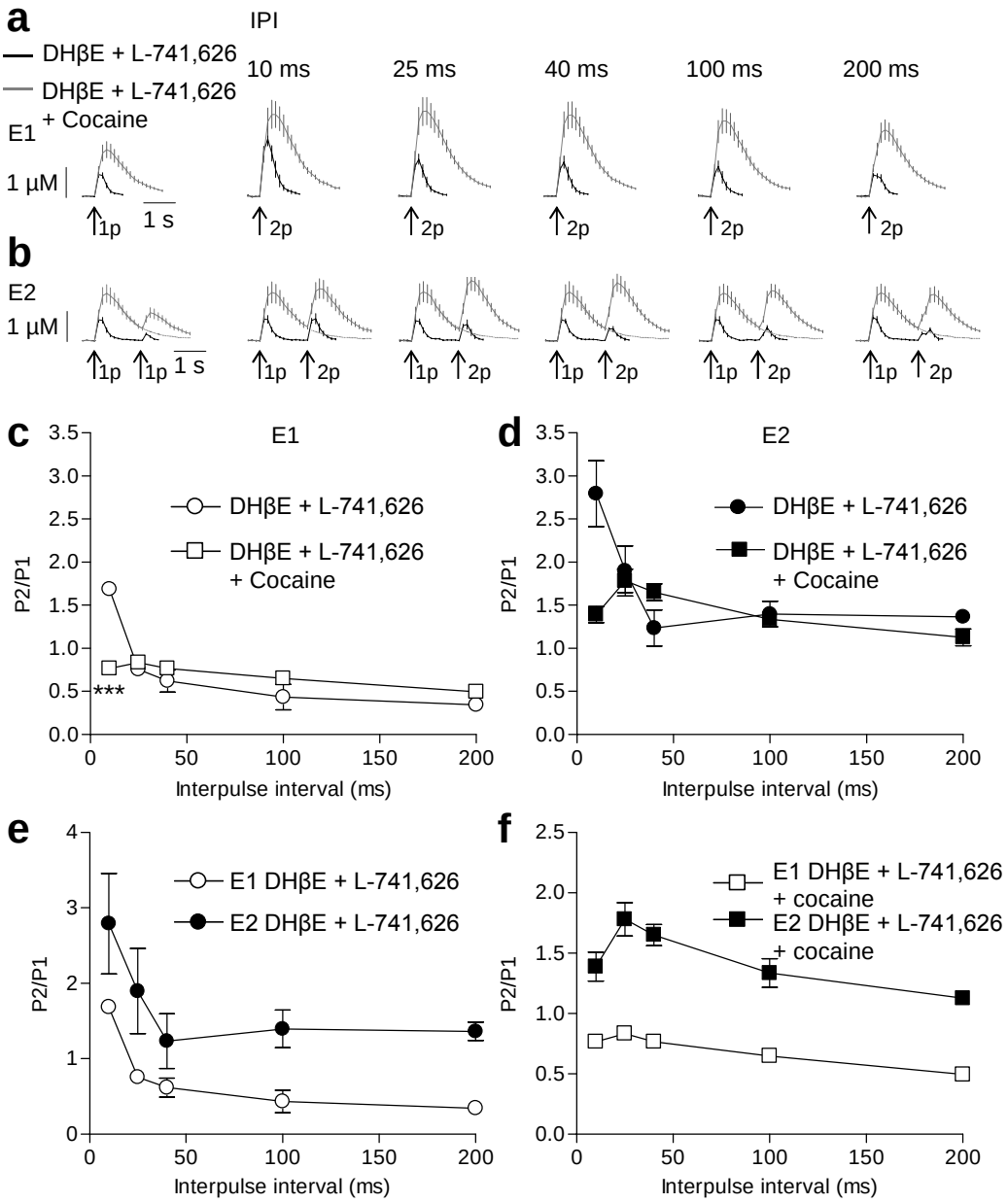

Figure 4.10. A background of DA release, and hence increased DA uptake, does not alter the short-term plasticity of DA release in a DAT-dependent manner (1.5 s interval). (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), at a first (E1) or second epoch (E2), with 1.5 s intervals between epochs, with D_2R inhibition (L-741,626, 1 µM) and nAChRs inhibited (DHβE, 1 µM), before and after cocaine (5 µM). **(c,d)** Mean P2/P1±SEM against IPI, with D_2R inhibition (L-741,626, 1 µM), and with DHβE (1 µM), before and after cocaine (5 µM) at E1 (c) or E2 (d). At E1, cocaine significantly decreased P2/P1 at 10 ms IPI (Two-way ANOVA, E1: Main effect of cocaine: $p > 0.05$, $F_{1,20}$=1.84, Main effect of IPI: $p < 0.001$ $F_{4,20}$=33.52, Interaction: $p < 0.001$, $F_{4,20}$=20.15, *post hoc* Bonferroni t test, $p < 0.001$ at 10 ms IPI; E2: Main effect of cocaine: $p > 0.05$, $F_{1,20}$=1.91, Main effect of IPI: $p > 0.05$, $F_{4,20}$=2.46, Interaction: $p > 0.05$, $F_{4,20}$=2.22, $n = 3$). **(e,f)** Mean P2/P1±SEM, against IPI at E1 and E2 with D_2R inhibition (L-741,626, 1 µM), and with DHβE (1 µM), in DHβE + L-741,626 (e), and with DHβE + L-741,626 + cocaine (5 µM) (f). There was a significant main effect of epoch on P2/P1 both before and after cocaine (Two-way ANOVA, DHβE: Main effect of epoch: $p < 0.001$, $F_{1,20}$=23.19, Main effect of IPI: $p < 0.01$ $F_{4,20}$=6.69, Interaction: $p > 0.05$, $F_{4,20}$=0.22; DHβE + Cocaine: Main effect of epoch: $p < 0.001$, $F_{1,20}$=207.23, Main effect of IPI: $p < 0.001$, $F_{4,20}$=10.83, Interaction: $p > 0.05$, $F_{4,20}$=1.65, $n = 3$).

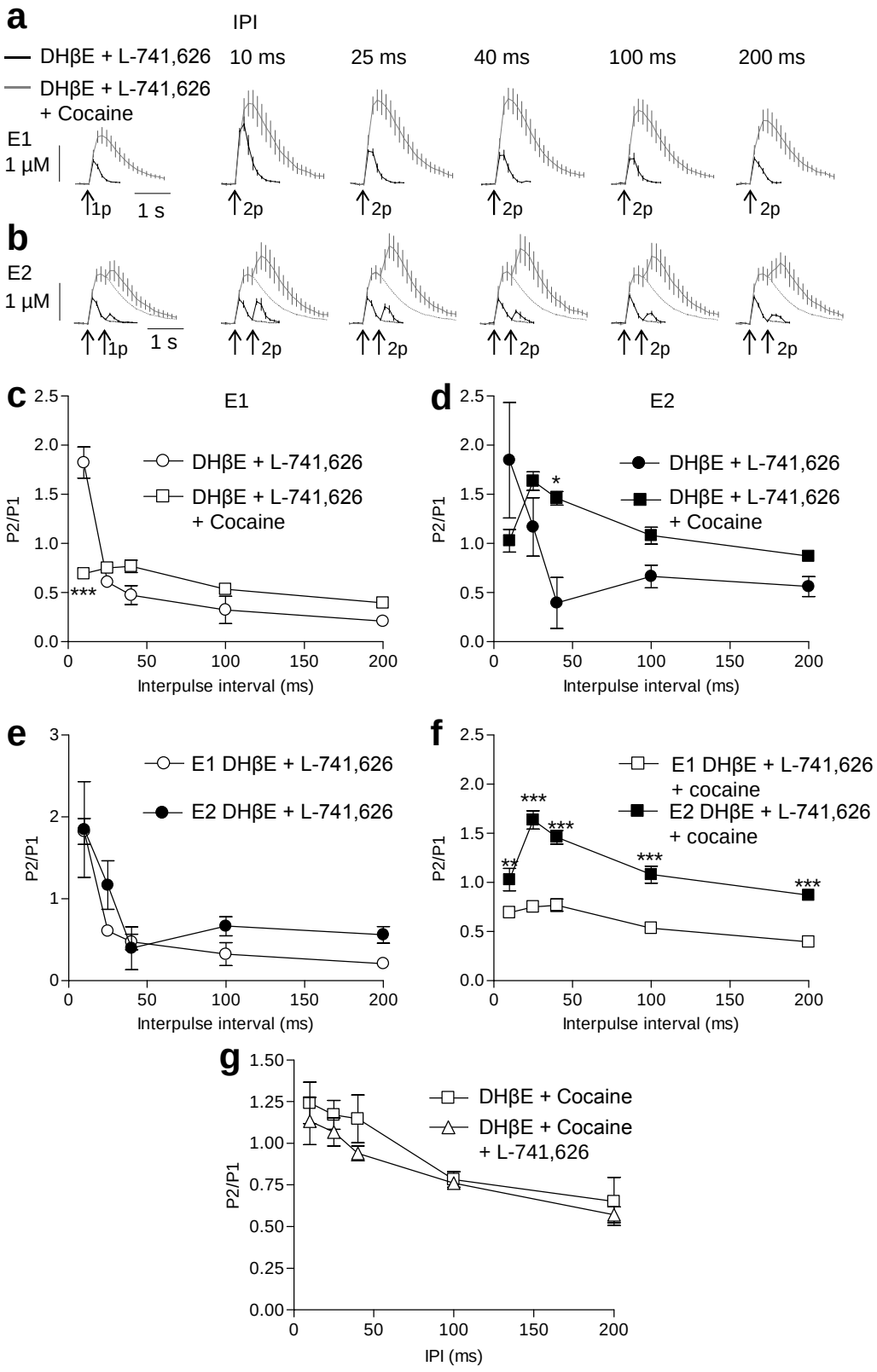
a
IPI
DHβE + L-741,626
DHβE + L-741,626
+ Cocaine
10 ms
25 ms
40 ms
100 ms
200 ms
E1
1 µM
1p
1 s
2p
b
E2
1 µM
c
E1
P2/P1
DHβE + L-741,626
DHβE + L-741,626
+ Cocaine
***
Interpulse interval (ms)
d
E2
*
e
E1 DHβE + L-741,626
E2 DHβE + L-741,626
f
E1 DHβE + L-741,626
+ cocaine
E2 DHβE + L-741,626
+ cocaine
**
***
g
DHβE + Cocaine
DHβE + Cocaine
+ L-741,626
IPI (ms)

Figure 4.11. A background of DA release, and hence increased DA uptake, does not alter the short-term plasticity of DA release in a DAT-dependent manner (0.5 s interval). (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms, E1 always 1p), at E1 (a) or E2 (b) with 0.5 s intervals between epochs, with D_2R inhibition (L-741,626, 1 µM), and nAChRs inhibited (DHβE, 1 µM), before and after cocaine (5 µM). **(c,d)** Mean P2/P1±SEM against IPI, at E1 (c) or E2 (d) with D_2R inhibition (L-741,626, 1 µM), and with DHβE (1 µM), before and after cocaine (5 µM). At both E1 and E2, cocaine significantly decreased the relationship between P2/P1 and IPI, decreasing P2/P1 at 10 ms IPI at E1 and increasing P2/P1 at 40 ms IPI at E2 (Two-way ANOVA, E1: Main effect of cocaine: $p > 0.05$, $F_{1,20}$=1.36, Main effect of IPI: $p < 0.001$ $F_{4,20}$=41.14, Interaction: $p < 0.001$, $F_{4,20}$=41.14, *post hoc* Bonferroni t test, $p < 0.001$ at 10 ms IPI; E2: Main effect of cocaine: $p > 0.05$, $F_{1,20}$=3.69, Main effect of IPI: $p < 0.05$, $F_{4,20}$=3.85, Interaction: $p < 0.05$, $F_{4,20}$=4.19, *post hoc* Bonferroni t test, $p < 0.05$ at 25 ms IPI $n = 3$). **(e,f)** Mean P2/P1±SEM against IPI before (e), and after cocaine (5 µM) (f) at E1 and E2, with D_2R inhibition (L-741,626, 1 µM) and with DHβE (1 µM). In cocaine, P2/P1 was significantly greater at E2 vs. E1 at all IPIs (Two-way ANOVA, DHβE: Main effect of epoch: $p < 0.05$, $F_{1,20}$=2.46, Main effect of IPI: $p < 0.001$ $F_{4,20}$=12.70, Interaction: $p > 0.05$, $F_{4,20}$=0.59; DHβE + Cocaine: Main effect of epoch: $p < 0.001$, $F_{1,20}$=198.82, Main effect of IPI: $p < 0.001$, $F_{4,20}$=24.44, Interaction: $p < 0.01$, $F_{4,20}$=5.09, *post hoc* Bonferroni t test, $p < 0.01$ at 10-100 ms IPI; $n = 3$). **(g)** Mean P2/P1±SEM against IPI after cocaine, before and after L-741,626, 1 µM. There was no significant effect of L-416,626 on P2/P1 (Two-way ANOVA, Main effect of L-741,626: $p > 0.05$, $F_{1,20}$=2.76, Main effect of IPI: $p < 0.001$ $F_{4,20}$=12.21, Interaction: $p > 0.05$, $F_{4,20}$=0.22; $n=3$).

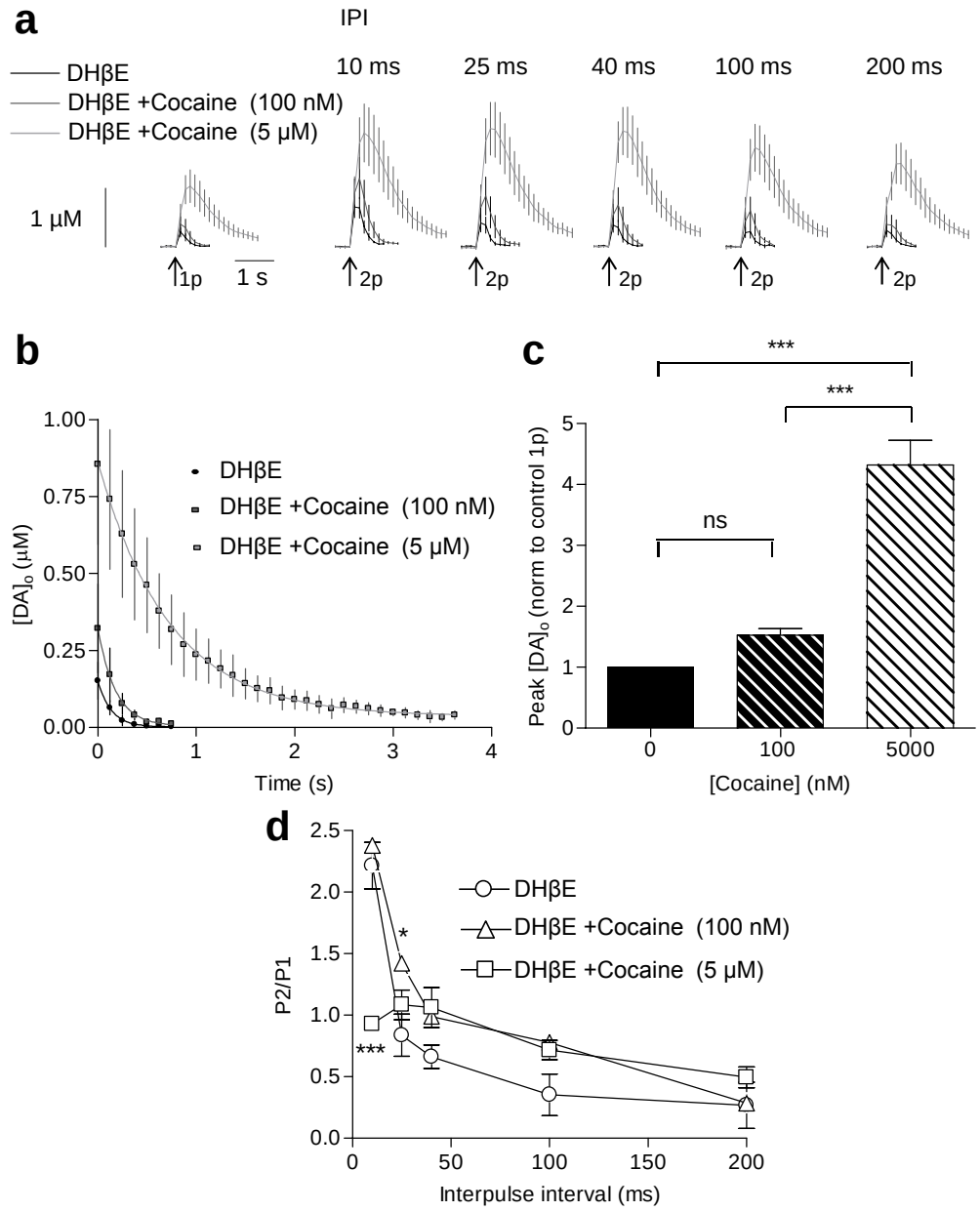

Figure 4.12. Low cocaine concentration shows that DAT effects in dlCPu on the short-term plasticity of release at short and longer IPIs are dissociable. (a) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow) (1p or 2p, IPI 10-200 ms), with nAChRs inhibited (DHβE, 1 µM), before and after cocaine (100 nM, 5 µM), in dlCPu. **(b)** Decay phases of profiles of $[DA]_o$±SEM evoked by 1p, against time after start of decay phase, with DHβE (1 µM), before and after cocaine (100 nM, 5 µM). Curve fits are one phase exponential decays, $R^2>0.94$. Rate constants of decay are significantly different between drug conditions (K=7.39 s^{-1} control, K=5.63 s^{-1} cocaine (100 nM), K=1.38 s^{-1} cocaine (5 µM), $p < 0.0001$ $F_{2,32}$=146.8; $n = 3$). **(c)** Mean peak $[DA]_o$±SEM evoked by 1p with DHβE (1 µM), with different cocaine concentrations, norm to peak $[DA]_o$ evoked by 1p in control conditions. 100 nM cocaine had no significant effect on peak 1p $[DA]_o$ but 5 µM cocaine significantly increased peak 1p $[DA]_o$ (One-way ANOVA, $p < 0.05$, $F_{2,8}$=53.63, *post hoc* Bonferroni t test, $p < 0.001$ for 5 µM cocaine; $n = 3$) **(d)** Mean P2/P1±SEM against IPI, with DHβE (1 µM), before and after cocaine (100 nM, 5 µM). 100 nM cocaine increased P2/P1 at 25 ms IPI, whereas 5 µM cocaine decreased P2/P1 at 10 ms IPI (Two-way ANOVA, Main effect of cocaine: $p < 0.001$, $F_{2,30}$=8.94, Main effect of IPI: $p < 0.001$ $F_{4,30}$=54.61, Interaction: $p < 0.001$, $F_{8,30}$=9.47, *post hoc* Bonferroni t test, $p < 0.05$ at 25 ms IPI in 100 nM cocaine and 10 ms IPI in 5 µM cocaine; $n = 3$).

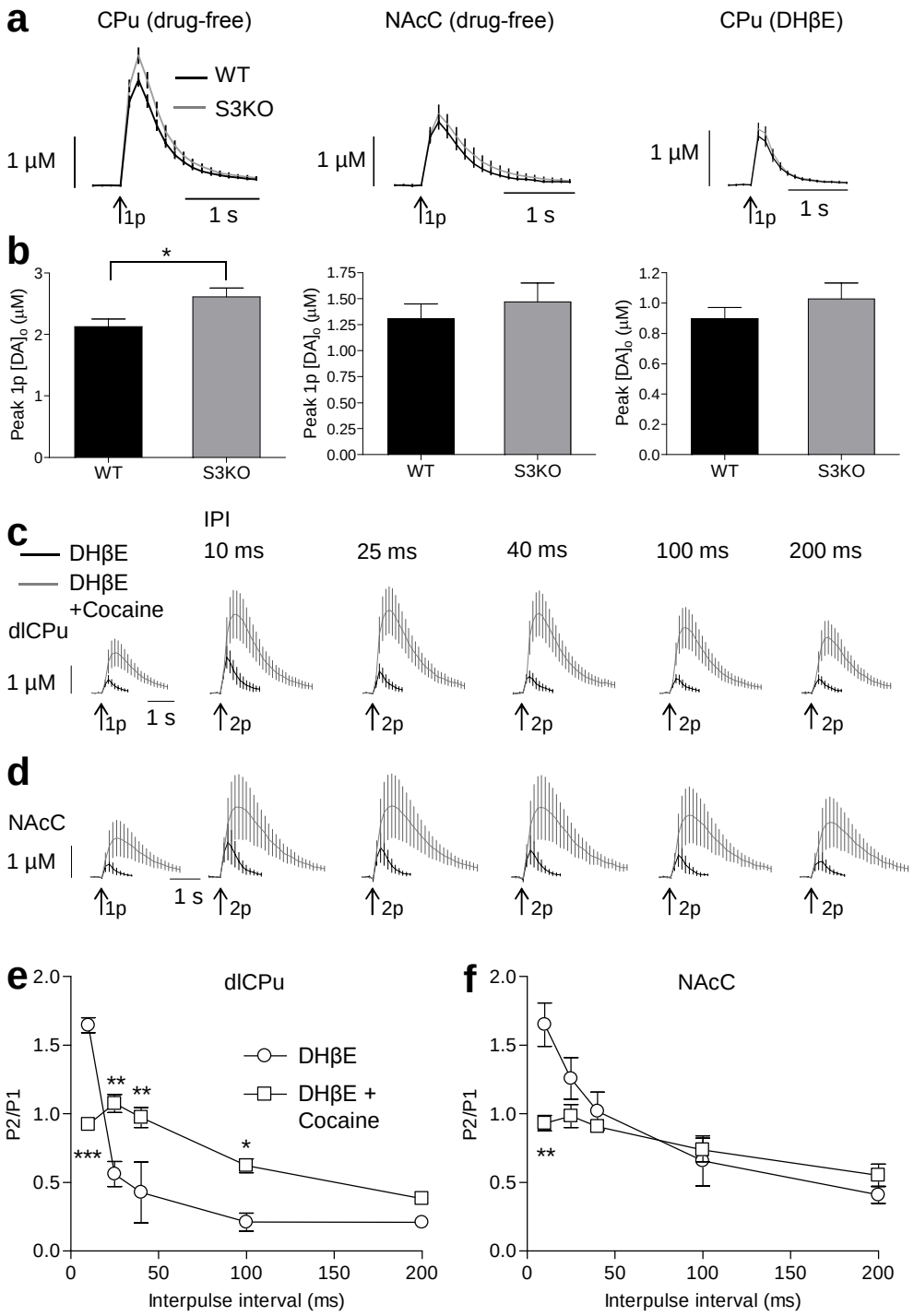

Figure 4.13. Effects of DAT inhibition on the short-term plasticity of DA release are not dependent on synapsin III. (a) Profiles of mean $[DA]_o$±SEM evoked by 1p, against time, in WT and S3KO in drug-free conditions in CPu (left), NAcC (middle) and with DHβE (1 µM) in CPu (right). **(b)** Mean peak $[DA]_o$±SEM evoked by 1p in WT and S3KO in drug-free conditions in CPu (left), NAcC (middle) and with DHβE (1 µM) in CPu (right). Peak $[DA]_o$ evoked by 1p was significantly higher in S3KO vs. WTs in drug-free conditions in CPu (Unpaired t test, $p < 0.05$, t = 2.50, df = 118; $n = 60$), but was not significantly different between genotypes in NAcC or with DHβE in CPu (Unpaired t test, $p > 0.05$, NAcC: t = 0.70, df = 46, dlCPu DHβE: t = 0.30, df = 32; $n = 20$-24). **(c,d)** Profiles of mean $[DA]_o$±SEM against time after stimulation (arrow) (1p or 2p, IPI 10-200 ms), with nAChRs inhibited (DHβE, 1 µM), with and without cocaine (5 µM), in dlCPu (c) and NAcC (d), in S3KO. **(e,f)** Mean P2/P1±SEM against IPI in dlCPu (e) and NAcC (f), with DHβE (1 µM), before and after cocaine (5 µM), in S3KO. Cocaine decreased P2/P1 at 10 ms IPI in both regions but increased it at 25-100 ms IPI in dlCPu (Two-way ANOVA, dlPCu: Main effect of cocaine: $p < 0.01$, $F_{1,20}$=10.37, Main effect of IPI: $p < 0.001$ $F_{4,20}$=36.42, Interaction: $p < 0.001$, $F_{4,20}$=16.99, *post hoc* Bonferroni t test, $p < 0.05$ at 10-100 ms IPI; NAcC: Main effect of cocaine: $p < 0.05$, $F_{1,20}$=6.02, Main effect of IPI: $p < 0.001$ $F_{4,20}$=16.33, Interaction: $p < 0.01$, $F_{4,20}$=4.60, *post hoc* Bonferroni t test, $p < 0.05$ at 10 ms IPI; $n = 3$).

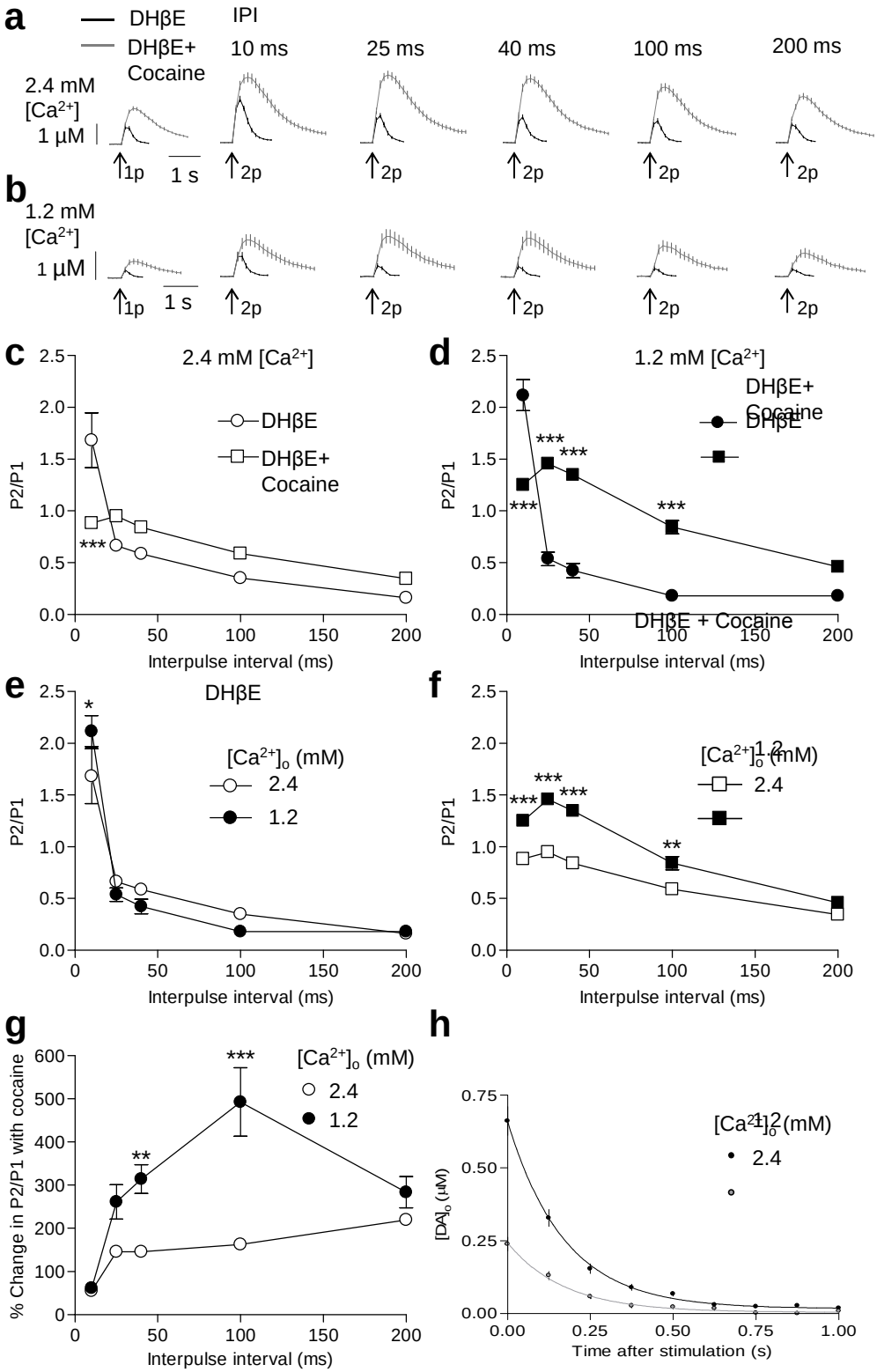
a
DHβE
DHβE+ Cocaine
IPI
10 ms
25 ms
40 ms
100 ms
200 ms
2.4 mM [Ca2+]
1 µM
1p
1 s
2p
b
1.2 mM [Ca2+]
1 µM
c
2.4 mM [Ca2+]
DHβE
DHβE+ Cocaine
P2/P1
Interpulse interval (ms)
d
1.2 mM [Ca2+]
DHβE
DHβE + Cocaine
e
DHβE
[Ca2+]o (mM)
2.4
1.2
f
g
% Change in P2/P1 with cocaine
h
[DA]o (µM)
Time after stimulation (s)

Figure 4.14. DAT effects on the short-term plasticity of DA release at long, but not short, IPIs are dependent on $[Ca^{2+}]_o$. (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), with nAChRs inhibited (DHβE, 1 µM), before and after cocaine (5 µM), in control (2.4 mM) $[Ca^{2+}]_o$ (a) or low (1.2 mM) $[Ca^{2+}]_o$ (b). **(c,d)** Mean P2/P1±SEM against IPI, with DHβE (1 µM), before and after cocaine (5 µM) in 2.4 (c) and 1.2 (d) mM $[Ca^{2+}]_o$. Cocaine significantly decreased P2/P1 at 10 ms IPI In both $[Ca^{2+}]_o$ and increased P2/P1 at 25-100 ms IPI in low $[Ca^{2+}]_o$ (Two-way ANOVA, 2.4 mM: Main effect of cocaine: $p > 0.05$, $F_{1,20}$=0.34, Main effect of IPI: $p < 0.001$ $F_{4,20}$=39.18, Interaction: $p < 0.001$, $F_{4,20}$=14.17, *post hoc* Bonferroni t test, $p < 0.05$ at 10 ms IPI; 1.2 mM: Main effect of cocaine: $p < 0.001$, $F_{1,20}$=75.26, Main effect of IPI: $p < 0.001$ $F_{4,20}$=113.45, Interaction: $p < 0.001$, $F_{4,20}$=56.86, *post hoc* Bonferroni t test, $p < 0.05$ at 10-100 ms IPI; $n = 3$). **(e,f)** Mean P2/P1±SEM against IPI in different $[Ca^{2+}]_o$ with DHβE (1 µM) (e) or with DHβE (1 µM) and cocaine (5 µM) (f). P2/P1 was increased in 1.2 vs. 2.4 mM $[Ca^{2+}]_o$ at 10 ms IPI in DHβE but at 10-100 ms IPI with DHβE + cocaine (Two-way ANOVA, DHβE: Main effect of $[Ca^{2+}]_o$: $p > 0.05$, $F_{1,20}$=0.00, Main effect of IPI: $p < 0.001$ $F_{4,20}$=91.71, Interaction: $p < 0.05$, $F_{4,20}$=3.06, *post hoc* Bonferroni t test, $p < 0.05$ at 10 ms IPI; DHβE+Cocaine: Main effect of $[Ca^{2+}]_o$: $p < 0.001$, $F_{1,20}$=159.23, Main effect of IPI: $p < 0.001$ $F_{4,20}$=113.91, Interaction: $p < 0.001$, $F_{4,20}$=7.41, *post hoc* Bonferroni t test, $p < 0.05$ at 10-100 ms IPI; $n = 3$). **(g)** Mean percentage change in P2/P1 with DHβE + cocaine vs. DHβE ±SEM, against IPI, in different $[Ca^{2+}]_o$. The effect of cocaine on P2/P1 was greater in 1.2 vs. 2.4 mM $[Ca^{2+}]_o$ at 40 and 100 ms IPI (Two-way ANOVA, DHβE: Main effect of $[Ca^{2+}]_o$: $p < 0.001$, $F_{1,20}$=43.55, Main effect of IPI: $p < 0.001$ $F_{4,20}$=18.14, Interaction: $p < 0.01$, $F_{4,20}$=7.13, *post hoc* Bonferroni t test, $p < 0.01$ at 40-100 ms IPI; $n = 3$).**(h)** Decay phases of profiles of $[DA]_o$±SEM evoked by 1p in different $[Ca^{2+}]_o$. Rate of decay was not significantly different between $[Ca^{2+}]_o$ ($p > 0.05$, $F_{1,17}$=1.54; $n = 3$).

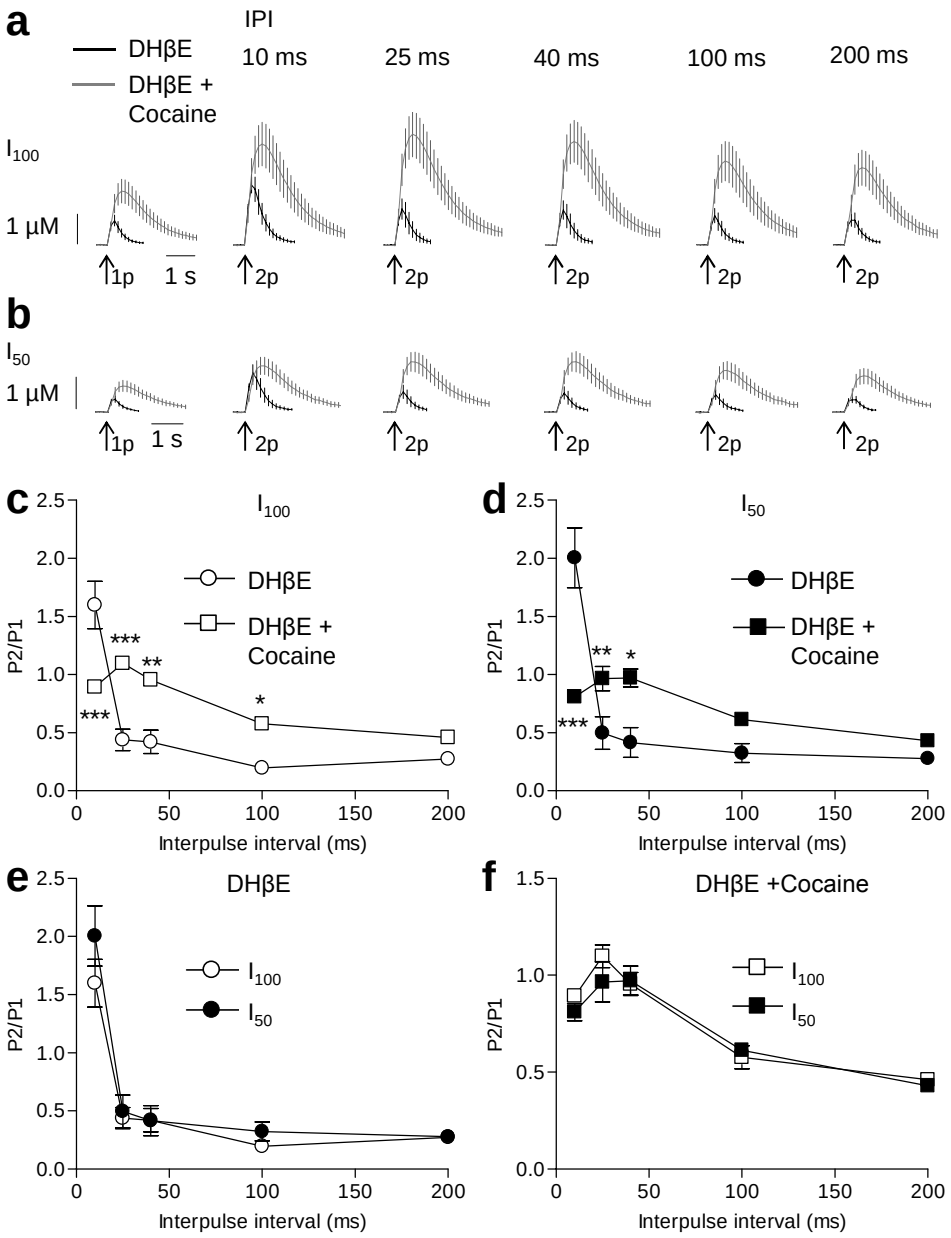

Figure 4.15. Reducing stimulation current does not mimic the effects of low $[Ca^{2+}]_o$ on the effect of DAT on P2/P1. (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow)(1p or 2p, IPI 10-200 ms), with nAChRs inhibited (DHβE, 1 µM), before and after cocaine (5 µM), with perimaximal current stimulation (I_{100}) (a) or stimulation at a current that approximately halves peak $[DA]_o$ evoked by 1p (I_{50}) (b). **(c,d)** Mean P2/P1±SEM against IPI, with DHβE (1 µM), before and after cocaine (5 µM) with I_{100} (c) and I_{50} (d) stimulation. At both currents, cocaine significantly decreased P2/P1 at 10 ms IPI but increased it at longer IPIs (Two-way ANOVA, I_{100}: Main effect of cocaine: $p < 0.001$, $F_{1,30}$=22.05, Main effect of IPI: $p < 0.001$ $F_{4,30}$=87.56, Interaction: $p < 0.001$, $F_{4,30}$=43.10, *post hoc* Bonferroni t test, $p < 0.05$ at 10-100 ms IPI; I_{50} : Main effect of cocaine: $p > 0.05$, $F_{1,30}$=0.11, Main effect of IPI: $p < 0.001$ $F_{4,30}$=85.32, Interaction: $p < 0.001$, $F_{4,30}$=62.72, *post hoc* Bonferroni t test, $p < 0.05$ at 10-40 ms IPI; $n = 4$). **(e,f)** Mean P2/P1±SEM against IPI with different current stimulations, in DHβE (e) or with DHβE + cocaine (5 µM) (f). There was no significant effect of current on the relationship between P2/P1 and IPI either in DHβE or with DHβE + cocaine (Two-way ANOVA, DHβE: Main effect of current: $p < 0.05$, $F_{1,30}$=4.70, Main effect of IPI: $p < 0.001$ $F_{4,30}$=149.73, Interaction: $p > 0.05$, $F_{4,30}$=1.40; DHβE + Cocaine: Main effect of current: $p < 0.05$, $F_{1,30}$=4.96, Main effect of IPI: $p < 0.001$ $F_{4,30}$=91.97, Interaction: $p > 0.05$, $F_{4,30}$=1.67; $n = 4$), although there was a trend towards increased P2/P1 at very short IPIs with low current.

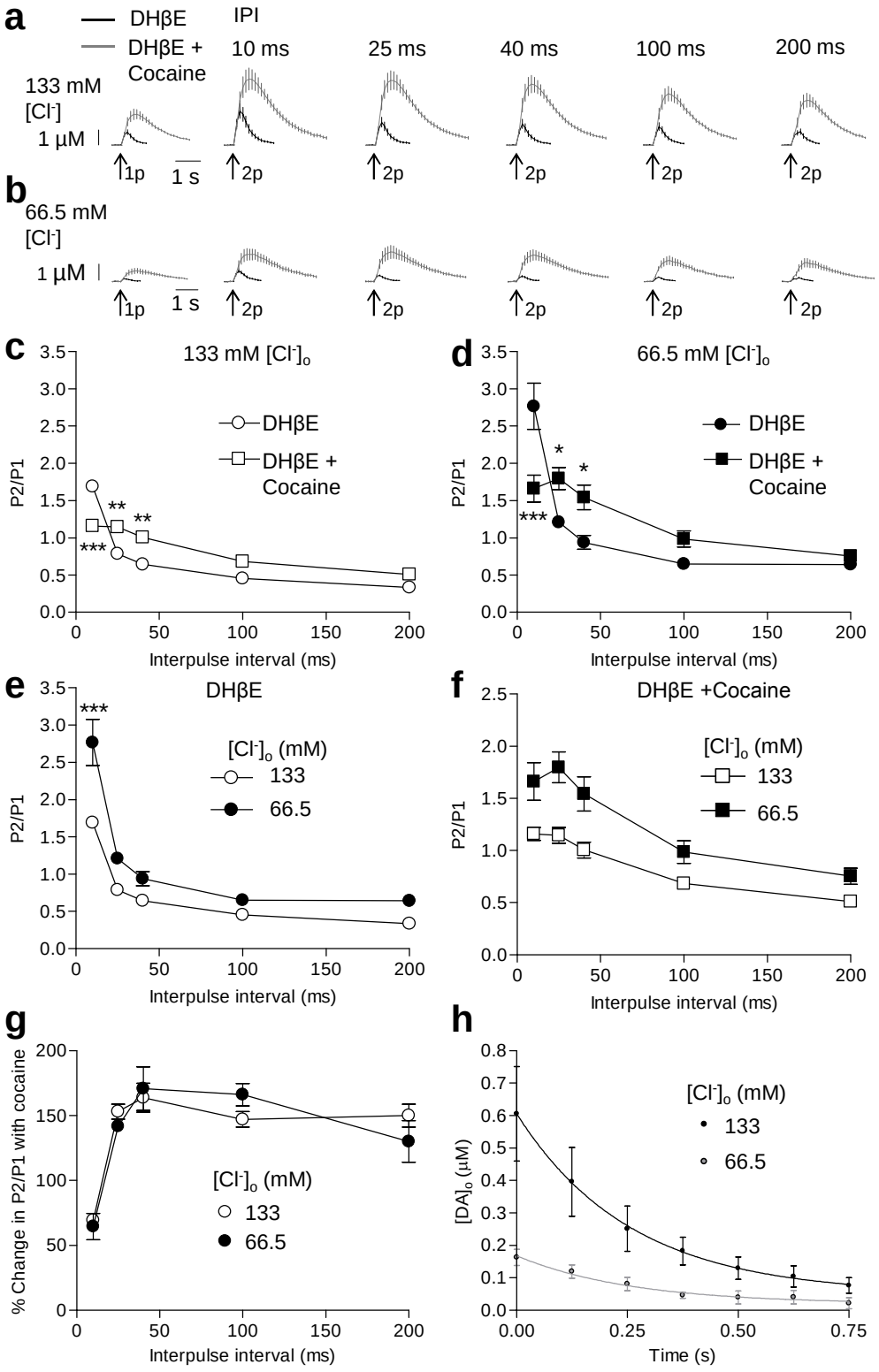
a
DHβE
DHβE + Cocaine
IPI
10 ms
25 ms
40 ms
100 ms
200 ms
133 mM [Cl-]
1 µM
1p
1 s
2p
b
66.5 mM [Cl-]
c
133 mM [Cl-]o
DHβE
DHβE + Cocaine
P2/P1
Interpulse interval (ms)
d
66.5 mM [Cl-]o
e
DHβE
[Cl-]o (mM)
133
66.5
f
DHβE +Cocaine
g
% Change in P2/P1 with cocaine
h
[DA]o (µM)
Time (s)

Figure 4.16. DAT effects on the short-term plasticity of DA release are not modulated by $[Cl^-]_o$. (a,b) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow) (1p or 2p, IPI 10-200 ms), with nAChRs inhibited (DHβE, 1 µM), before and after cocaine (5 µM), in control (133 mM) $[Cl^-]_o$ (a) or low (33.5 mM) $[Cl^-]_o$ (b). **(c,d)** Mean P2/P1±SEM against IPI, with DHβE (1 µM), before and after cocaine (5 µM) in 133 (c) and 66.5 (d) mM $[Cl^-]_o$. In both $[Cl^-]_o$, cocaine significantly decreased P2/P1 at 10 ms IPI but increased it at 25-40 ms IPIs (Two-way ANOVA, 133 mM: Main effect of cocaine: $p < 0.01$, $F_{1,20}$=10.44, Main effect of IPI: $p < 0.001$ $F_{4,20}$=90.05, Interaction: $p < 0.001$, $F_{4,20}$=20.59, *post hoc* Bonferroni t test, $p < 0.01$ at 10-40 ms IPI; 66.5 mM: Main effect of cocaine: $p > 0.05$, $F_{1,20}$=1.38, Main effect of IPI: $p < 0.001$ $F_{4,20}$=35.56, Interaction: $p < 0.001$, $F_{4,20}$=12.04, *post hoc* Bonferroni t test, $p < 0.05$ at 10-40 ms IPI; $n = 3$). **(e,f)** Mean P2/P1±SEM against IPI in DHβE (e) or with DHβE + cocaine (5 µM) (f). In DHβE, decreasing $[Cl^-]_o$ increased P2/P1 at 10 ms IPI. With DHβE + cocaine, there was no significant interaction between $[Cl^-]_o$ and IPI but there was a main effect of $[Cl^-]_o$ with P2/P1 higher in 66.5 vs 133 mM $[Cl^-]_o$ (Two-way ANOVA, DHβE: Main effect of $[Cl^-]_o$: $p < 0.001$, $F_{1,20}$=43.42, Main effect of IPI: $p < 0.001$ $F_{4,20}$=83.09, Interaction: $p < 0.01$, $F_{4,20}$=5.29, *post hoc* Bonferroni t test, $p < 0.001$ at 10 ms IPI; DHβE + Cocaine: Main effect of $[Cl^-]_o$: $p < 0.001$, $F_{1,20}$=41.72, Main effect of IPI: $p < 0.001$ $F_{4,20}$=22.97, Interaction: $p > 0.05$, $F_{4,20}$=1.19; $n = 3$).**(g)** Mean percentage change in P2/P1 with cocaine vs. control ±SEM, against IPI, with DHβE (1 µM), in different $[Cl^-]_o$. The effect of cocaine on P2/P1 was not modulated by $[Cl^-]_o$ (Two-way ANOVA, Main effect of $[Cl^-]_o$ $p > 0.05$, $F_{1,20}$=0.10, Main effect of IPI: $p < 0.001$ $F_{4,20}$=31.38, Interaction: $p > 0.05$, $F_{4,20}$=1.17; $n = 3$).**(h)** Decay phases of profiles of $[DA]_o$±SEM evoked by 1p, in different $[Cl^-]_o$. Rate of decay was not significantly different between $[Cl^-]_o$ ($p > 0.05$, $F_{1,5}$=5.02, $n = 3$).

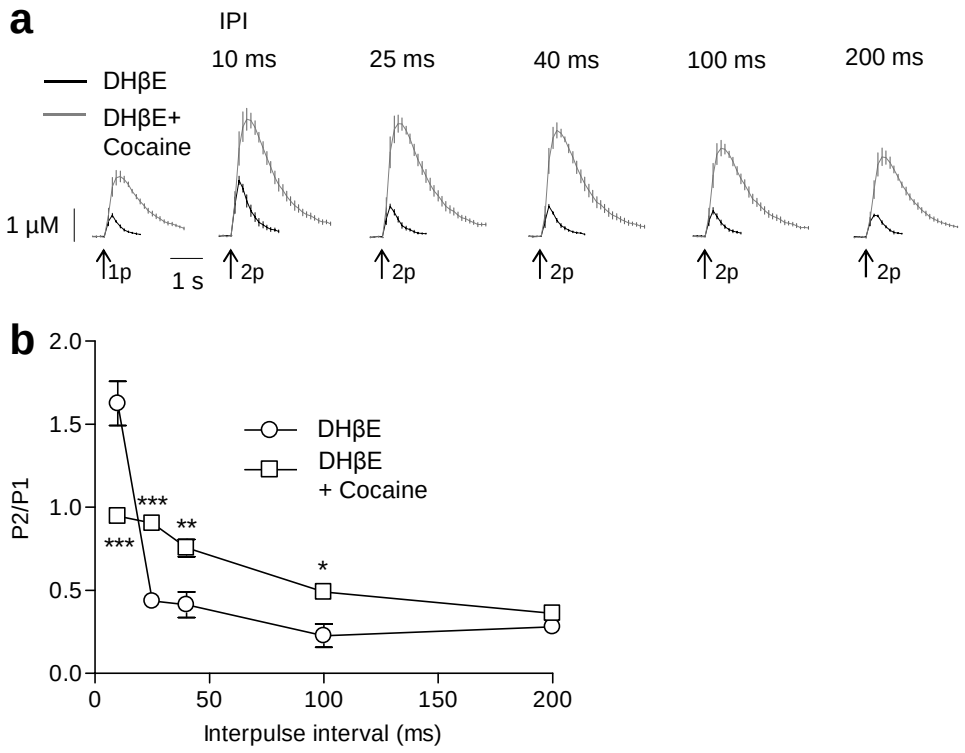

Figure 4.17. Effects of DAT inhibition on the short-term plasticity of DA release are similar at physiological temperatures. (a) Profiles of mean $[DA]_o$±SEM against time after single or paired pulse stimulations (arrow) (1p or 2p, IPI 10-200 ms) with nAChRs inhibited (DHβE, 1 µM), before and after cocaine (5 µM), in dlCPu, at 37°C. **(b)** Mean P2/P1±SEM against IPI, with DHβE (1 µM), before and after cocaine (5 µM), at 37°C. Cocaine significantly decreased P2/P1 at 10 ms IPI but increased it at 25-100 ms IPI (Two-way ANOVA, Main effect of temperature $p < 0.05$, $F_{1,20}$=6.58, Main effect of IPI: $p < 0.001$ $F_{4,20}$=87.88, Interaction: $p < 0.001$, $F_{4,20}$=30.16, *post hoc* Bonferroni t test, $p < 0.05$ at 10-100 ms IPI; $n = 3$).

5. Regional differences in the indirect DAT control of the short-term plasticity of DA release, when nAChRs are active

5.1 Introduction

In the previous chapter I explored a previously unidentified role for the DAT in controlling the short-term plasticity of DA release, when nAChRs were inhibited. Previous work in our lab has demonstrated a different role for the DAT in controlling DA release and the short-term plasticity of release, via control of D_2R activation, when nAChRs are active (Clements *et al. In preparation*). In this chapter I have further explored this indirect role of the DAT, particularly looking at differences in this effect between dorsal and ventral striatum, which may be significant in the actions of drugs of abuse.

5.1.1 An indirect role for the DAT in controlling DA release, via control of D_2R activation

When two single pulse stimulations are applied to a striatal slice, separated by short intervals, DA release evoked by the second stimulation (2^{nd} epoch, E2) is significantly lower than that evoked by the first (1^{st} epoch, E1), as discussed in chapter 1.2.2.3. This is partly due to the short-term depression of DA release, but also partly due to D_2R activation (Kennedy *et al.* 1992; Clements *et al. In preparation*). Previous work in our lab has shown that, in addition to this decrease in 1p release, when nAChRs are not inhibited, the short-term plasticity of DA release is altered at E2 compared to E1. At E2, the sensitivity of DA release to high frequency burst activity is increased; that is, the ratio of $[DA]_o$ evoked by high frequency burst stimulation to that evoked by a single pulse or low frequency stimulation, is increased at E2 vs. E1. This effect is dependent on D_2Rs, as it is greatly attenuated by D_2R antagonists (Clements *et al. In preparation*). It is proposed that D_2R activation suppresses initial DA release and relieves the short-term depression of release, increasing the sensitivity of release to high frequency burst stimulation (Clements *et al. In preparation*).

Previous work in our lab has demonstrated that DAT inhibitors like cocaine can amplify and prolong this D_2R-mediated effect on DA release and the short-term plasticity of release. DAT inhibitors increase the amplitude and lifetime of extracellular DA signals, increasing and prolonging D_2R activation and hence D_2R-mediated effects on DA release (Clements *et al. In preparation*). In this way, on a background of activity, cocaine alters the short-term plasticity of release, increasing the sensitivity of DA release to burst vs. low frequency stimulation (**Fig. 5.1**). This would be hypothesised to increase the contrast between striatal DA signals evoked by reward-related burst activity and tonic striatal DA. This would be expected to increase detection of reward-related DA signals by MSNs, promoting plasticity in MSNs and hence behavioural reinforcement. Therefore, this effect of cocaine on DA release plasticity may contribute to the reinforcing effects of the drug.

5.1.2 Dorsal vs. ventral striatum

In this chapter I have explored whether this D_2R-dependent effect of cocaine on the short-term plasticity of DA release differs in efficacy between dlCPu and NAcC. Many studies have shown that cocaine preferentially increases extracellular DA concentrations in the ventral vs. the dorsal striatum (Carboni *et al.* 1989; Cass *et al.* 1992; Kuczenski and Segal 1992; Wu *et al.* 2001). This may be significant to its behavioural effects since DA in the ventral, as opposed to the dorsal, striatum is thought to be significant for the initial reinforcing effects of drugs of abuse(Everitt and Wolf 2002). However, this preferential effect in the ventral striatum seems paradoxical since cocaine binds to the DAT and inhibits uptake with similar efficacy throughout the striatum (Boja and Kuhar 1989; Cass *et al.* 1992; Jones *et al.* 1995), and there is in fact greater DAT density in the dorsal vs. ventral striatum (Marshall *et al.* 1990). A number of possible mechanisms have been suggested to underlie this preferential increase of DA in the ventral striatum with cocaine, ranging from the complex effects of lower DA P_r combined with lower uptake in the ventral striatum (Wu *et al.* 2001) to a preferential increase in the frequency of release events in the

ventral striatum (Aragona *et al.* 2008). However, it remains incompletely explained. In this chapter I have explored whether the D_2R-dependent effect of cocaine on the sensitivity of DA release to burst stimulation occurs with greater efficacy in the NAcC vs. dlCPu.

5.1.3 Which population of D_2Rs?

It is not currently known which population of D_2Rs is significant in mediating the effects of cocaine on DA release and the short-term plasticity of release. DA release can be driven either by activity in DA neurons themselves, or by ACh released from ChIs acting at nAChRs on DA axons (Threlfell *et al.* 2012). D_2Rs are located both on DA terminals and on ChIs, and hence are ideally placed to modulate both these components of DA release. D_2R modulation of either component may be hypothesised to modulate the short-term plasticity of DA release. D_2 autoreceptors on DA terminals have a well-characterised role in decreasing subsequent DA release, over a time course of around 250 ms to several seconds (Benoit-Marand *et al.* 2001; Phillips *et al.* 2002) (**Fig. 5.2 a**), via modulation of presynaptic VGCCs and K^+ channels (Cardozo and Bean 1995; Congar *et al.* 2002; Martel *et al.* 2011). As discussed in chapter 3, this decrease in release may be predicted to modulate the short-term plasticity of DA release at short IPIs, increasing the sensitivity of release to burst stimulation. D_2Rs on ChIs decrease ChI firing and reduce ACh release (DeBoer and Abercrombie 1996; Yan *et al.* 1997; Pisani *et al.* 2000) by modulating Ca^{2+} currents (Yan *et al.* 1997), VGSC currents (Maurice *et al.* 2004) and the I_h (Deng *et al.* 2007). Since ACh strongly controls the short-term plasticity of release via nAChRs on DA terminals (Rice and Cragg 2004; Zhang and Sulzer 2004), a decrease in ACh release would be predicted to alter DA release plasticity, again increasing the sensitivity of release to burst stimulation. Therefore, although it has not yet been directly demonstrated, D_2Rs on ChIs are hypothesised to strongly control DA release and the short-term plasticity of release (**Fig. 5.2 b**). Indeed, a recent report showed that when D_2Rs are selectively knocked out of DA neurons, the D_2R agonist quinpirole can still greatly

decrease striatal DA release (Anzalone *et al.* 2012), suggesting a strong role for D_2 heteroceptors in controlling DA release. D_2Rs on ChIs seem a prime candidate for mediating this response.

It is not currently known which population of D_2Rs, or changes in which component of DA release, are significant in mediating the D_2R-dependent effects of cocaine on the short-term plasticity of DA release. Given the strong effect of nAChRs, relative to P_r, on the short-term plasticity of DA release, presented in chapter 3, D_2R modulation of ACh release and hence nAChR activation seems a strong candidate. Indeed, changes in nAChR signalling have been suggested to underlie changes in the short-term plasticity of DA release induced by other drugs of abuse, such as nicotine and opioids (Rice and Cragg 2004; Britt and McGehee 2008). In this chapter, I have used an optogenetic approach to dissect out the D_2R control of DA release evoked by DA neuron activity and by ACh, to explore which component may mediate the effect of cocaine on the sensitivity of DA release to burst stimulation, and have tested the hypothesis that D_2R control of DA release may also vary with striatal region.

5.2 Methods

Methods were carried out as described in chapter 2, unless otherwise described here.

5.2.1 Animals

Experiments using electrical stimulation have used adult male C57Bl6/J mice. Experiments using an optogenetic approach have used adult male $DAT^{Cre/+}$ or $ChAT^{Cre/+}$ mice transfected with AAV5-ChR2, as described in chapter 2.3.2. All procedures were carried out in accordance with UK Home Office project licence and local guidelines.

5.2.2 Slice preparation and FCV

Acute striatal slices were prepared and FCV was carried out as described in chapter 2.

5.2.3 Stimulation and experimental design

DA release was evoked either using electrical or light stimulation, as described in chapter 2.3. In all experiments in this chapter, the short-term plasticity of $[DA]_o$ was explored by applying alternating single pulse (1p) and burst (4p) stimulations. Burst sensitivity of DA release was defined as the ratio of peak $[DA]_o$ evoked by 4p to that evoked by 1p (4p:1p). Electrical stimulation experiments used burst stimulations of 100 Hz frequency, to maximize 4p:1p (Exley *et al.* 2007; Threlfell *et al.* 2010) and hence allow for the easiest detection of subtle changes in this parameter. However, ChR2 cannot follow such high stimulation frequencies (Nagel *et al.* 2003; Tsai *et al.* 2009). Therefore in optogenetic experiments using light stimulation, burst stimulations of 25 Hz were used.

In experiments investigating the effect of cocaine on DA release on a background of activity, 4p:1p was explored both at an initial stimulation (1^{st} epoch, E1) and at a second stimulation (2^{nd} epoch, E2), 3 s after E1. Alternating 1p and 4p stimulations were applied at both E1 and E2, but all E2 stimulations followed a 1p E1 stimulation.

In concentration response experiments, increasing concentrations of drugs were washed on and alternating 1p and 4p stimulations applied until $[DA]_o$ was stable (15-20 mins) before data was included in analysis.

5.2.4 Data analysis

Data were acquired and analysed as described in chapter 2.2.2. The rate of DA uptake was analysed using one phase exponential decay curve fits of the falling phases of mean $[DA]_o$ profiles evoked by 1p, in GraphPad Prism. Concentration responses were compared using sigmoidal dose response (variable slope) curve fits in GraphPad Prism.

5.2.5 Drugs

DHβE and quinpirole hydrochloride were purchased from Tocris Biosciences or Ascent Scientific and cocaine hydrochloride was purchased from Sigma-Aldrich. Drugs were dissolved in distilled water to make stock aliquots 1000x final concentrations and stored at -20^{o}C. The final concentration was made up in aCSF immediately before use.

5.3 Results

In this chapter I have explored the D_2R-dependent effects of cocaine on the sensitivity of DA release to high frequency burst stimulation on a background of activity in dlCPu vs. NAcC. I have then begun to tease apart the D_2R control of different components of DA release, which mediate this effect of cocaine.

Throughout this chapter control conditions are drug-free, with nAChRs not inhibited unless described otherwise.

5.3.1 Cocaine promotes the burst sensitivity of DA release with a greater efficacy in NAcC vs. dlCPu

To explore the burst sensitivity of DA release on a background of activity, two electrical stimulations separated by 3 s (E1 and E2) were applied. Both E1 and E2 stimulations consisted of alternating single pulse (1p) and burst (4p, 100 Hz) stimulations (E1 1p only shown, **Fig. 5.3 a,b**). This stimulation protocol was applied in a range of cocaine concentrations (0-10 μM) in dlCPu and NAcC, to compare the effects of cocaine on DA release and burst sensitivity of release between regions (**Fig. 5.3 a,b**).

As expected from its function as a DAT inhibitor, increasing concentrations of cocaine increasingly prolonged the duration of $[DA]_o$ profiles (**Fig. 5.3 a,b**). The decay phases of $[DA]_o$ profiles evoked by 1p at E1 were curve fit as exponential decays ($R^2 > 0.93$; $n = 3$) (**Fig. 5.3 c,d**,

limited cocaine concentrations shown for clarity), and the rate constant of decay plotted against concentration of cocaine (**Fig. 5.3 e**). In both regions, cocaine increased the rate constant of decay in a concentration-dependent manner, consistent with a concentration-dependent decrease in DA uptake (curve fits sigmoidal dose-response curves (variable-slope), $R^2 > 0.95$; $n = 3$). Consistent with previous reports (Jones *et al.* 1995; Cragg *et al.* 2000), at all concentrations of cocaine, the rate of decay was slower in NAcC than in dlCPu. However, there was no significant difference in the $LogEC_{50}$ of the effect of cocaine on the rate constant of decay between regions ($p > 0.05$; $n = 3$) (**Fig. 5.3 e**), consistent with reports that cocaine binds to the DAT with similar affinity, and has similar effects on the K_m of the DAT, in dorsal and ventral striatum (Cass *et al.* 1992; Jones *et al.* 1995).

The effects of cocaine on DA release were first explored at E1. Cocaine modulated peak $[DA]_o$ evoked by 1p at E1 similarly in both striatal regions (**Fig. 5.3 f**). At concentrations up to 1 µM, cocaine increased peak $[DA]_o$ evoked by 1p (**Fig. 5.3 f**), consistent with its known effects of blocking DA uptake and promoting DA release (Venton *et al.* 2006). At concentrations greater than 1 µM, peak $[DA]_o$ evoked by 1p decreased with increasing cocaine concentration (**Fig. 5.3 f**). This may be due to increased tonic activation of D_2Rs and hence decreased DA release with increasing concentrations of cocaine, or changes in DA vesicle organisation or release with prolonged DAT inhibition. At E1, the burst sensitivity of DA release was low in all concentrations of cocaine in both regions, with 4p:1p approximately 1.1 (**Fig. 5.3 g**). This is indicative of the strong short-term depression of DA release (Rice and Cragg 2004; Zhang and Sulzer 2004), see chapter 3) and consistent with the minimal effects of DAT inhibition on the short-term plasticity of DA release at E1, when nAChRs are active (Cragg 2003; Zhang *et al.* 2009).

The effect of cocaine on $[DA]_o$ evoked by E2 stimulations was then explored. At E2, increasing concentrations of cocaine increased peak $[DA]_o$ evoked by 1p at concentrations up to 500 nM, then decreased it at higher concentrations, in both striatal regions (**Fig. 5.3 h,i**). In contrast, peak

$[DA]_o$ evoked by 4p was increased by increasing cocaine concentrations up to 2-5 µM, and only decreased at the highest concentrations (**Fig. 5.3 h,i**). Therefore, at E2, cocaine increased 4p:1p in a concentration-dependent manner in both striatal regions (**Fig. 5.3 h,i,j**) (curve fits are sigmoidal dose-response (variable slope) curves, dlCPu: $R^2 = 0.86$, NAcC: $R^2 = 0.40$; $n = 3$). Previous work in our lab has demonstrated that this suppression of E2 1p, and promotion of E2 burst sensitivity by cocaine, can be greatly attenuated by a D_2R antagonist (Clements *et al. In preparation*). Therefore, it is hypothesised that increasing concentrations of cocaine prolong E1 extracellular DA signals in a concentration-dependent manner, and hence increase and prolong D_2R activation, leading to a decrease in subsequent, E2, release. This also relieves the short-term depression of DA release, promoting the burst sensitivity of release at E2.

The concentration dependence of the cocaine effect on the burst sensitivity of $[DA]_o$ at E2 was compared in dlCPu vs. NAcC (**Fig. 5.3 j**). The $LogEC_{50}$ of this effect of cocaine was significantly different between regions ($R^2 > 0.88$, $p < 0.001$; $n = 3$) with an EC_{50} of 2.37 µM cocaine in dlCPu and 1.04 µM in NAcC. Therefore, cocaine promotes the burst sensitivity of DA release on a background of activity, with a greater efficacy in NAcC compared to dlCPu.

Several potential mechanisms for this regional difference can be ruled out based on the data above. The above results showed that cocaine had similar effects on the rate of DA uptake and the peak $[DA]_o$ evoked by 1p in both regions, ruling out regional differences in the effect of cocaine on DA uptake or 1p DA release. Due to the prolongation of DA profiles, in higher concentrations of cocaine $[DA]_o$ remained above baseline 3 s after E1, at the time of E2 (**Fig. 5.3 a,b**). However, $[DA]_o$ 3 s after E1 was greater in dlCPu than NAcC in all concentrations of cocaine (**Fig. 5.3 k**). This rules out the regional difference in the D_2R-dependent effect of cocaine being due to a greater level of $[DA]_o$, and hence increased D_2R activation, at the time of E2 in NAcC vs. dlCPu. Therefore, it was hypothesised that the regional difference in the D_2R-mediated effect of

cocaine on the burst sensitivity of DA release may be due to regional differences in the D_2R control of DA release and the short-term plasticity of release.

5.3.2 D_2R control of DA release evoked by DA neuron activity occurs with similar efficacy in dlCPu and NAcC

To test the hypothesis that there are regional differences in the D_2R control of DA release, the concentration response of the D_2R agonist quinpirole has been compared between striatal regions. As discussed previously, striatal DA release can be driven both by DA neuron activity and by ACh released from ChIs acting at nAChRs on DA terminals (Threlfell *et al.* 2012). Both components of DA release are likely to be controlled by D_2Rs, since D_2Rs are expressed both on DA neuron terminals and on ChIs, and, as described above in 5.1.3, D_2R control of either component of DA release may be hypothesised to mediate the effects of cocaine on the short-term plasticity of DA release. Therefore, the D_2R control of both components of DA release has been explored. An optogenetic approach has been used to dissect these two components of DA release, using $DAT^{Cre/+}$ mice to selectively express ChR2 in DA neurons and hence evoke DA release by DA neuron activity, and $ChAT^{Cre/+}$ mice to selectively express ChR2 in ChIs and hence drive ACh-evoked DA release. The concentration response of quinpirole has been compared between striatal regions in both lines of mice.

Alternating 1p and 4p, 25Hz light stimulations were applied to slices from $DAT^{Cre/+}$ mice, in a range of quinpirole concentrations (0-5 µM), in dlCPu and NAcC (**Fig. 5.4 a,b**). Quinpirole decreased peak $[DA]_o$ evoked by both 1p and 4p in a concentration-dependent manner in both striatal regions (**Figs. 5.3 c,d**) (curve fits are sigmoidal dose-response (variable slope) curves, $R^2 > 0.76$; $n = 3$). The concentration-dependence of the effect of quinpirole on peak $[DA]_o$ evoked by 1p was similar between regions, but the $LogEC_{50}$ was slightly different (**Fig. 5.3 e**)(curve fits are sigmoidal dose-response (variable slope) curves, $R > 0.99$; $p < 0.01$; $n = 3$), with an EC_{50} of 38.4

nM in dlCPu and 32.0 nM in NAcC. Therefore, D_2R control of DA release evoked by DA neuron activity in $DAT^{Cre/+}$ occurs with very slightly greater efficacy in NAcC vs. dlCPu.

In dlCPu, quinpirole had no effect on 4p:1p (**Fig. 5.3 c,f**) but in NAcC, quinpirole increased 4p:1p in a concentration-dependent manner (**Fig. 5.3 d,f**) (curve fits are sigmoidal dose-response (variable slope) curves, dlCPu: $R^2 = 0$, NAcC: $R^2 = 0.40$; $n = 3$). Therefore, D_2Rs control the sensitivity of DA release to 25 Hz burst activity in DA neurons in NAcC but not in dlCPu. This may be consistent with some results shown in chapter 3, which suggested that when nAChRs are not active, changes in initial DA release have no effect on subsequent release in dlCPu at 25 Hz (an IPI of 40 ms) but that in NAcC, initial and subsequent release may be weakly inversely related at this IPI (see **Figs. 3.8, 3.9**). Alternatively, it is feasible that this difference between NAcC and dlCPu reflects completely different downstream actions of D_2Rs on DA signalling in the two regions. The use of higher stimulation frequencies, at which initial release controls the short-term plasticity of release similarly in dlCPu and NAcC (see chapter 3), might identify which is the case. However, ChR2 only reliably follows frequencies of light stimulation up to 25 Hz. For this reason, electrical stimulation in slices from WT animals, using the nAChR antagonist DHβE to inhibit ACh-evoked DA release, was used to further explore D_2R control of DA release evoked by DA neuron activity.

Alternating 1p and 4p, 100 Hz electrical stimulations were applied to slices from WT mice, in increasing concentrations of quinpirole (0-5 μM), in DHβE (1 μM), in dlCPu and NAcC (**Fig. 5.5 a,b**). Quinpirole decreased peak $[DA]_o$ evoked by both 1p and 4p in a concentration-dependent manner in both regions (**Fig. 5.5 c,d**) (curve fits are sigmoidal dose-response (variable slope) curves, $R^2 > 0.85$; $n = 3$). The concentration-dependence of quinpirole inhibition of peak $[DA]_o$ evoked by 1p was compared in dlCPu and NAcC and the $LogEC_{50}$ was not significantly different between regions, with EC_{50} in dlCPu 20.3 nM compared to 18.7 nM in NAcC (**Fig. 5.5 e**) (curve fits are sigmoidal dose-response (variable slope) curves, $R^2 > 0.97$, $p > 0.05$; $n = 3$). Therefore, D_2R control of electrically evoked DA release in WTs, with nAChRs inhibited, is similar between

striatal regions, although the trend was in the same direction as in $DAT^{Cre/+}$ animals, with greater efficacy in NAcC vs. dlCPu.

In both regions, quinpirole increased 4p:1p in a concentration dependent manner (**Fig. 5.4 c,d,f**) (curve fits are sigmoidal dose response (variable slope) curves, $R^2 > 0.88$). This suggests that the effects of D_2 autoreceptor signalling, to decrease initial release, relieve depression and promote subsequent release and hence burst sensitivity, are similar in both striatal regions. The lack of quinpirole effect on burst sensitivity in dlCPu in optogenetic experiments most likely reflects the lack of relationship between initial and subsequent release with an IPI of 40 ms in this region, but a slight relationship between these parameters in NAcC (see chapter 3). The $LogEC_{50}$ of this effect of quinpirole on 4p:1p was not significantly different between regions (**Fig.5.5 f**) ($R^2>0.62$; $p > 0.05$; $n = 3$). This suggests that the regional differences in the efficacy of cocaine on the burst sensitivity of DA release are not due to regional differences in the efficacy of the D_2R control of the burst sensitivity of DA release evoked by DA neuron activity.

5.3.3 D_2Rs control ACh-evoked DA release with greater efficacy in NAcC than in dlCPu

D_2R control of DA release evoked by ACh was then explored. Light stimulation of slices from $ChAT^{Cre/+}$ mice transfected with AAV5-ChR2 was used to selectively evoke activity in ChIs, driving DA release via the actions of ACh at nAChRs on DA terminals (Threlfell *et al.* 2012).

Alternating 1p and 4p, 25 Hz light stimulations were applied in increasing concentrations of quinpirole (0-500 nM) (**Fig. 5.6 a,b**). Quinpirole decreased peak $[DA]_o$ evoked by both 1p and 4p in a concentration-dependent manner in both regions (**Fig. 5.6 c,d**) (curve fits are sigmoidal dose response (variable slope) curves, $R^2 > 0.95$; $n = 3$). The concentration-dependence of the quinpirole inhibition of 1p release was compared in dlCPu and NAcC (**Fig. 5.6 e**). The $LogEC_{50}$ of this effect was significantly different between regions ($p < 0.01$; $n = 3$) with an EC_{50} of 23.8 nM in

dlCPu and 16.4 nM in NAcC (**Fig. 5.6 e**). Therefore, D_2R control of ACh-evoked DA release occurs with a greater efficacy in NAcC vs. dlCPu.

There was no burst sensitivity of ACh-evoked DA release; 4p:1p was approximately 1 in all quinpirole concentrations (**Fig. 5.6 c,d,f**). This is consistent with the known insensitivity of ACh-evoked DA release to burst stimulation and different frequencies of stimulation (Threlfell *et al.* 2012).

5.4 Discussion

In this chapter I have explored regional differences in a role of the DAT in controlling the short-term plasticity of DA release when nAChRs are active, which occurs via controlling the amplitude and lifetime extracellular striatal DA signals, and hence controlling D_2R activation, which in turn modulates DA release. It was found that this mechanism occurs with different efficacy in NAcC vs. dlCPu. Since this effect depends on D_2Rs, I then explored regional differences in the D_2R control of DA release evoked both by DA neuron activity and by ACh from striatal ChIs.

5.4.1 Cocaine promotes the burst sensitivity of DA release with greater efficacy in NAcC vs. dlCPu

Previous work in our lab has shown that, on a background of prior DA release, when nAChRs are active, cocaine or other DAT inhibitors can alter the short-term plasticity of DA release, promoting the burst sensitivity of DA release. This effect is dependent on D_2Rs (Clements *et al. In preparation*). It is proposed that cocaine prolongs extracellular DA signals by blocking reuptake, which increases activation of D_2Rs on both DA terminals and on ChIs. This results in the suppression of subsequent DA release, relieving short-term depression and hence increasing the burst sensitivity of DA release. The data presented in this chapter showed that this effect of cocaine occurs with greater efficacy in NAcC vs. dlCPu, that is that lower concentrations of

cocaine preferentially increase the burst sensitivity of DA release in NAcC vs. dlCPu. Several potential mechanisms for this regional difference can be ruled out. Cocaine inhibition of DA uptake and modulation of 1p release occurred with similar efficacy in both striatal regions, consistent with previous reports (Cass *et al.* 1992; Jones *et al.* 1995). The level of extracellular DA present above baseline 3 s after E1 stimulation, that is at the time of the E2 stimulation, was greater in dlCPu than NAcC at all concentrations of cocaine, most likely due to the fact that 1p release is greater on average in dlCPu than NAcC, and cocaine similarly affects 1p release and uptake in the two regions. Therefore, the regional difference in the efficacy of cocaine on the burst sensitivity of release cannot be attributed to a greater effect of cocaine on uptake or initial DA release, or greater extracellular levels of DA and hence enhanced D_2R activation at E2, in NAcC vs. dlCPu. It was therefore hypothesised that there might be differences in the D_2R control of DA release in the two striatal regions. Hence the D_2R control of DA release was explored further.

5.4.2 D_2R control of DA release evoked by DA neuron activity occurs with a similar efficacy in dlCPu and NAcC

The D_2R control of DA release evoked both by DA neuron activity and by ACh was compared in dlCPu and NAcC. The D_2R control of DA release evoked by DA neuron activity was explored, firstly using an optogenetic approach to selectively activate DA neurons, and secondly using electrical stimulation in the presence of a nAChR antagonist to inhibit ACh-evoked DA release. It is most likely that the only population of D_2Rs involved in controlling this component of DA release is D_2 autoreceptors on DA terminals.

D_2R activation using the D_2R agonist quinpirole decreased DA release evoked by 1p in a concentration-dependent manner in both optogenetic and electrical experiments. This is consistent with the well-characterised role of D_2 autoreceptors in the negative feedback control of DA release (Schmitz *et al.* 2002; Schmitz 2003). This effect occurred with similar efficacy in

both striatal regions; in the optogenetic experiments the EC_{50} was slightly lower in NAcC vs. dlCPu, but in the electrical experiments there was no significant difference, although the trend was in the same direction. Therefore, a slight difference in the efficacy of autoreceptor control of DA release may contribute to the different efficacy of cocaine in the two striatal regions, but appears too slight to fully account for this.

In addition to the disparity in different regional efficacy between optogenetic and electrical experiments, the EC_{50} of the quinpirole inhibition of 1p release was greater in optogenetic than electrical experiments, ~35 nM compared to ~20 nM. There are several possible reasons for these disparities between the optogenetic and electrical experiments. Firstly, in the optogenetic experiments only DA axons are activated, whereas in electrical experiments all neuronal types in the slice are activated. Therefore, in optogenetic experiments only D_2 autoreceptors on DA terminals will be able to control DA release. In electrical experiments, while the effects of ACh acting via nAChRs were blocked using a nAChR antagonist, glutamate and GABA transmission were not blocked. It is therefore feasible that in electrical experiments D_2Rs located on the terminals of glutamate afferents, or on striatal GABAergic neurons, modulate glutamate or GABA transmission, and indirectly affect DA release. This is unlikely however, as glutamate and GABA transmission are not thought to influence DA release evoked by the stimulation protocols used here (Cragg 2003; Zhang and Sulzer 2003). Also, the D_2R-dependent effect of cocaine on E2 release has previously been shown to be unaffected by glutamate and GABA antagonists, suggesting that D_2R mediated control of DA release does not occur via changes in glutamate or GABA transmission (Clements *et al*. in preparation). Another possible reason for differences between optogenetic and electrical experiments is that $DAT^{Cre/+}$ animals show an approximately 17% decrease in DAT expression (Bäckman *et al.* 2006). This may cause subtle changes in striatal DA signalling, driving compensatory changes in the control of DA release. Although there is no overall change in striatal D_2R expression in $DAT^{Cre/+}$ animals (Bäckman *et al.* 2006), this does not rule out that there may be changes specifically in D_2 autoreceptor expression, or in the pathways

downstream of D_2Rs. A different possible reason for differences between optogenetic and electrical experiments is that DA release driven by light activation of ChR2 may be controlled differently by D_2Rs compared to release driven by electrical stimulation. For example, P_r may differ slightly between optogenetic and electrical experiments since in electrical experiments a perimaximal stimulation current was used, but in some optogenetic experiments in dlCPu, a perimaximal light intensity could not be reached (see chapter 2.4). Alternatively, ChR2 activation may drive greater Ca^{2+} entry than electrical stimulation, which may alter P_r or vesicle trafficking, or interact with D_2R-driven changes in Ca^{2+} or K^+ conductances, in a manner which alters the D_2R control of DA release.

In addition to its effects on release evoked by 1p, quinpirole increased the burst sensitivity of DA release evoked by DA neuron activity in a concentration-dependent manner, in both regions in electrical experiments and in NAcC in optogenetic experiments. This likely occurs because D_2 autoreceptor activation suppresses initial DA release, which in the absence of nAChR activity relieves short-term depression of release at short IPIs and hence enhances the contrast between DA release evoked by high frequency burst stimulation and that evoked by a single pulse. This effect on burst sensitivity mimics the D_2R-dependent effect of cocaine on E2 DA release. Therefore, increased D_2 autoreceptor activation likely contributes to the enhanced burst sensitivity of DA release at E2 in the presence of cocaine. However, in electrical experiments, this quinpirole-induced increase in burst sensitivity occurred with similar efficacy in dlCPu and NAcC. Therefore, differences in the autoreceptor control of burst sensitivity do not appear to contribute to the differential efficacy in this effect of cocaine between striatal regions.

In optogenetic experiments in $DAT^{Cre/+}$ animals, quinpirole increased burst sensitivity in NAcC but not in dlCPu. This could feasibly reflect different downstream effects of autoreceptor activation in the two regions. However, this seems unlikely since quinpirole did increase the burst sensitivity of DA release in dlCPu in electrical experiments using higher stimulation frequencies,

indicating that this mechanism does exist in dlCPu. Instead, the lack of effect of quinpirole on burst sensitivity in dlCPu in optogenetic experiments most likely reflects the different time course of the effect of P_r on the short-term plasticity of DA release in dlCPu vs. NAcC seen in chapter 3. In optogenetic experiments, due to the kinetics of ChR2, only stimulations at frequencies up to 25 Hz can be used. As presented in chapter 3, changes in initial DA release only appear to control the short-term plasticity of release, and hence burst sensitivity, at the shortest IPIs or highest frequencies; at longer IPIs or lower frequencies DA release exhibits release-independent plasticity. The frequencies over which this occurs may be slightly different in dlCPu and NAcC, with NAcC showing a release-dependent component of plasticity at slightly lower frequencies than dlCPu. Therefore, at the lower burst frequency used in optogenetic experiments, the suppression of initial release may alter subsequent release and hence burst sensitivity in NAcC, but not in dlCPu. This is interesting to note since it may be argued that this lower frequency is more representative of DA neuron burst firing *in vivo*. Therefore, this may be a further reason, in addition to the greater efficacy of cocaine in NAcC, to suggest that *in vivo* cocaine may promote the burst sensitivity of DA release more in NAcC than in dlCPu.

The results presented here suggest that D_2 autoreceptor control of DA release evoked by DA neuron activity is largely similar in dlCPu and NAcC. D_2 autoreceptors suppress DA release with a similar efficacy in both regions, but may have a slightly greater efficacy in NAcC. This suppression of initial release relieves short term depression and promotes the burst sensitivity of release at high frequencies in both regions. This likely contributes to the effects of cocaine on burst sensitivity at E2. This occurs at a lower frequency in NAcC than in dlCPu, suggesting that, *in vivo*, cocaine may promote burst sensitivity more in NAcC than in dlCPu. However, at high frequencies the efficacy of this effect is similar in both regions, so differential autoreceptor control of burst sensitivity does not account for the regional difference in efficacy of cocaine on the burst sensitivity of release.

5.4.3 D_2R control of ACh-evoked DA release occurs with greater efficacy in NAcC vs. dlCPu

Since D_2 autoreceptor control of DA release cannot completely account for the different efficacy of the cocaine effect on burst sensitivity in NAcC vs. dlCPu, the D_2R control of the ACh-evoked component of DA release was then explored. An optogenetic approach was used to selectively activate ChIs and drive ACh-evoked DA release. To date, little is known about the control of ACh-evoked DA release. However, it is hypothesised that ACh-evoked DA release is controlled both by D_2Rs on ChIs, which act to decrease ChI firing and ACh release (DeBoer and Abercrombie 1996; Pisani *et al.* 2000), and by D_2 autoreceptors on DA terminals.

Quinpirole decreased ACh-evoked DA release in a concentration-dependent manner, with greater efficacy in NAcC vs. dlCPu, with the EC_{50} approximately 50% greater in dlCPu vs. NAcC. The burst sensitivity of ACh-evoked release was very low, with 4p releasing no more DA than 1p. Quinpirole had no effect on this low burst sensitivity of ACh-evoked DA release in either striatal region. This is consistent with the reported insensitivity of ACh-evoked DA release to stimulation number and frequency in all conditions (Threlfell *et al.* 2012). The mechanisms underlying this are uncertain but may involve desensitisation of nAChRs, very strong depression of ACh release or changes in the DA terminal following nAChR activation which inhibit subsequent nAChR-evoked release. However, despite the insensitivity of ACh-evoked DA release to burst stimulation, suppression of ACh release and nAChR activation would be hypothesised to promote the burst sensitivity of DA release evoked by DA neuron activity, as discussed previously (Rice and Cragg 2004; Zhang and Sulzer 2004). Therefore, differential D_2R control of ACh-evoked DA release may contribute to the regional differences in efficacy of the effect of cocaine on burst sensitivity at E2. It would be interesting to explore this further, perhaps using channelrhodopsins activated by different wavelengths of light to selectively activate ChIs, then DA neurons, to explore the burst sensitivity of DA release evoked by DA neuron activity following changes in ChI activation.

5.4.4 Possible mechanisms of regional differences in D_2R efficacy

There was very little regional difference in efficacy of D_2R control of DA release evoked by DA neuron activity, but a greater difference in control of ACh-evoked release. D_2R control of release evoked by DA neuron activity will only involve D_2 autoreceptors on DA terminals, whereas D_2R control of ACh-evoked release most likely involves both autoreceptors on DA terminals and heteroceptors on ChIs. It is conceivable that autoreceptors control release evoked by DA neuron activity and release evoked by ACh via different pathways or mechanisms. Therefore, regional differences in the D_2R control of ACh-evoked DA release may feasibly be caused by regional differences in D_2 autoreceptor control of ACh-evoked release, even though there is very little difference in the autoreceptor control of release evoked by DA neuron activity. However, it seems more likely that the regional difference in the efficacy of D_2R control of ACh-evoked DA release is due to a regional difference in the efficacy of heteroceptors on ChIs, possibly in combination with the very subtle differences in autoreceptor control.

There are many possible explanations for a regional difference in the efficacy of D_2 heteroceptors on ChIs. ChIs in the striatum show a distinct spatiotemporal pattern of development, with development and maturation occurring in a lateral-medial direction in the striatum (Phelps *et al.* 1989; Chen *et al.* 2010). It is therefore possible that ChIs form distinct regional populations within the striatum with different protein expression patterns. Firstly, D_2R expression levels in ChIs may differ between dorsal and ventral striatum. D_2R expression in the striatum has been reported to be uneven or patchy, most likely reflecting differential D_2R expression in patch and matrix striatal compartments, but is grossly similar in dorsal and ventral striatum (Boyson *et al.* 1986; Bouthenet *et al.* 1991; Yung *et al.* 1995). However, the large majority of striatal D_2R expression detected in the above studies will be on neurons other than ChIs, including on MSNs and DA axons. Therefore, such studies are unlikely to detect subtle regional differences in D_2R expression on ChIs, so it is possible that ChIs in the dlCPu and NAcC express different levels of

D_2Rs. Secondly, there are two possible splice variants of D_2Rs, a long and short variant (D_2L and D_2S), which have distinct downstream effects (Guiramand *et al.* 1995). It is not currently known which variant is expressed in ChIs; it is possible that levels of these splice variants vary with striatal region and contribute to the regional difference in efficacy. Alternatively, there may be regional differences in the coupling of D_2Rs to the downstream G-protein-dependent signalling pathways, or differences in expression of the many effector proteins controlled by D_2R signalling in ChIs, such as VGSCs , VGCCs or hyperpolarisation activated Na^+ channels (Yan *et al.* 1997; Maurice *et al.* 2004) (Deng *et al.* 2007). Subtle differences in the expression patterns of any of these proteins or the coupling between them, may contribute to the regional differences in efficacy of D_2R control of ACh-evoked DA release. Finally, regional differences in D_2R control of ACh-evoked DA release may be caused, not by differences in the coupling of D_2Rs to ACh release, but by regional variations in ACh signalling in the striatum, for example differences in ACh tone or nAChR expression levels. It would be interesting to explore further if this is the case, since it would have implications for a number of striatal functions, and the actions of the addictive drug nicotine.

5.5 Implications and summary

It has been shown previously that, on a background of DA release, which may mimic the ongoing tonic DA release found *in vivo*, the DAT plays an indirect role in controlling the short-term plasticity of DA release. The DAT shapes the extracellular DA signal, limiting its sphere of influence and lifetime. DAT inhibitors, such as cocaine, prolong striatal DA signals, increasing D_2R activation. This increased D_2R activation promotes the contrast between DA signals evoked by high frequency burst firing, which occurs *in vivo* in response to reward-related or salient events, and those evoked by low frequency tonic firing. This will have downstream effects on information processing and plasticity in MSNs, and may contribute to the reinforcing effects of

such drugs. The initial reinforcing effects of cocaine depend on DA signalling specifically in the ventral striatum (Everitt and Wolf 2002). Additionally, cocaine is known to preferentially increase DA in the ventral vs. dorsal striatum (Carboni *et al.* 1989; Cass *et al.* 1992; Kuczenski and Segal 1992; Wu *et al.* 2001) but the mechanism underlying this remains uncertain. In this chapter it was found that the D_2R-dependent effect of cocaine on DA release plasticity occurs with greater efficacy in NAcC vs. dlCPu, most likely due to a greater efficacy of the D_2R control of ACh-evoked DA release. It is also interesting to note that at the lower frequency of stimulation used in optogenetic experiments, which may be more representative of the frequency of DA neuron burst firing reported *in vivo*, D_2 autoreceptor activation promoted burst sensitivity in NAcC but not dlCPu. These stronger effects of cocaine on the burst sensitivity of DA release in the NAcC vs. dlCPu reveal interesting differences in the control of DA release between striatal regions, and may contribute to the preferential increase in DA in the ventral vs. dorsal striatum reported in response to cocaine administration, which may be significant in the behavioural effects of the drug.

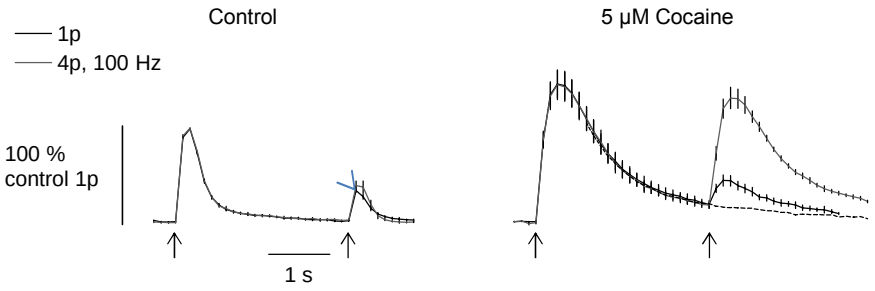

Figure 5.1 Cocaine increases the burst sensitivity of DA release on a background of activity, when nAChRs are active. In control conditions, at E2, the burst sensitivity of DA release is low, that is there is little contrast between $[DA]_o$ evoked by 1p and that evoked by 4p, 100Hz. With 5 µM cocaine, the burst sensitivity of DA release at E2 is much greater, that is the contrast between $[DA]_o$ evoked by 1p and that evoked by 4p, 100Hz is large.

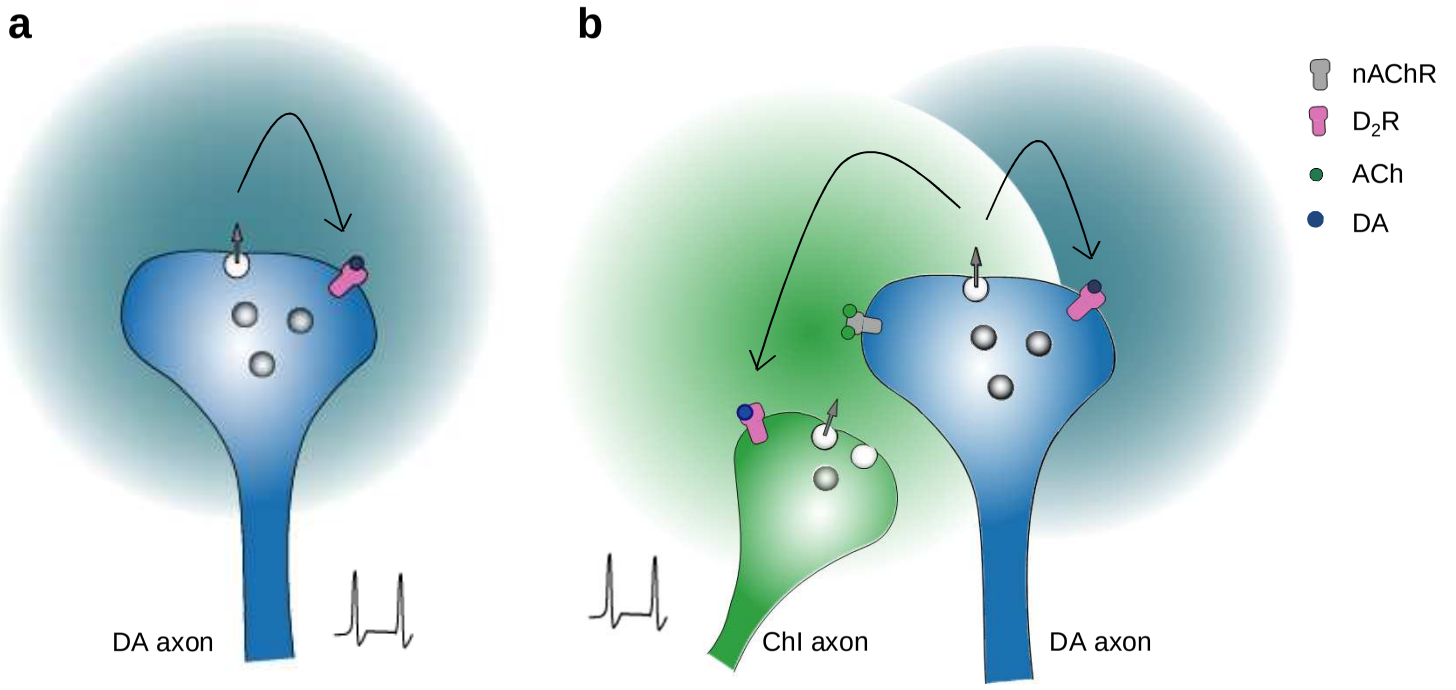

Figure 5.2. D_2Rs are ideally placed to control DA release evoked both by DA neuron activity and by nAChRs **(a)** DA release suppresses subsequent release evoked by DA neuron activity via D_2 autoreceptors on DA terminals. **(b)** DA release is predicted to decrease subsequent ACh-evoked DA release, both via D_2 autoreceptors on DA terminals and via D_2Rs on ChIs which reduce ACh release.

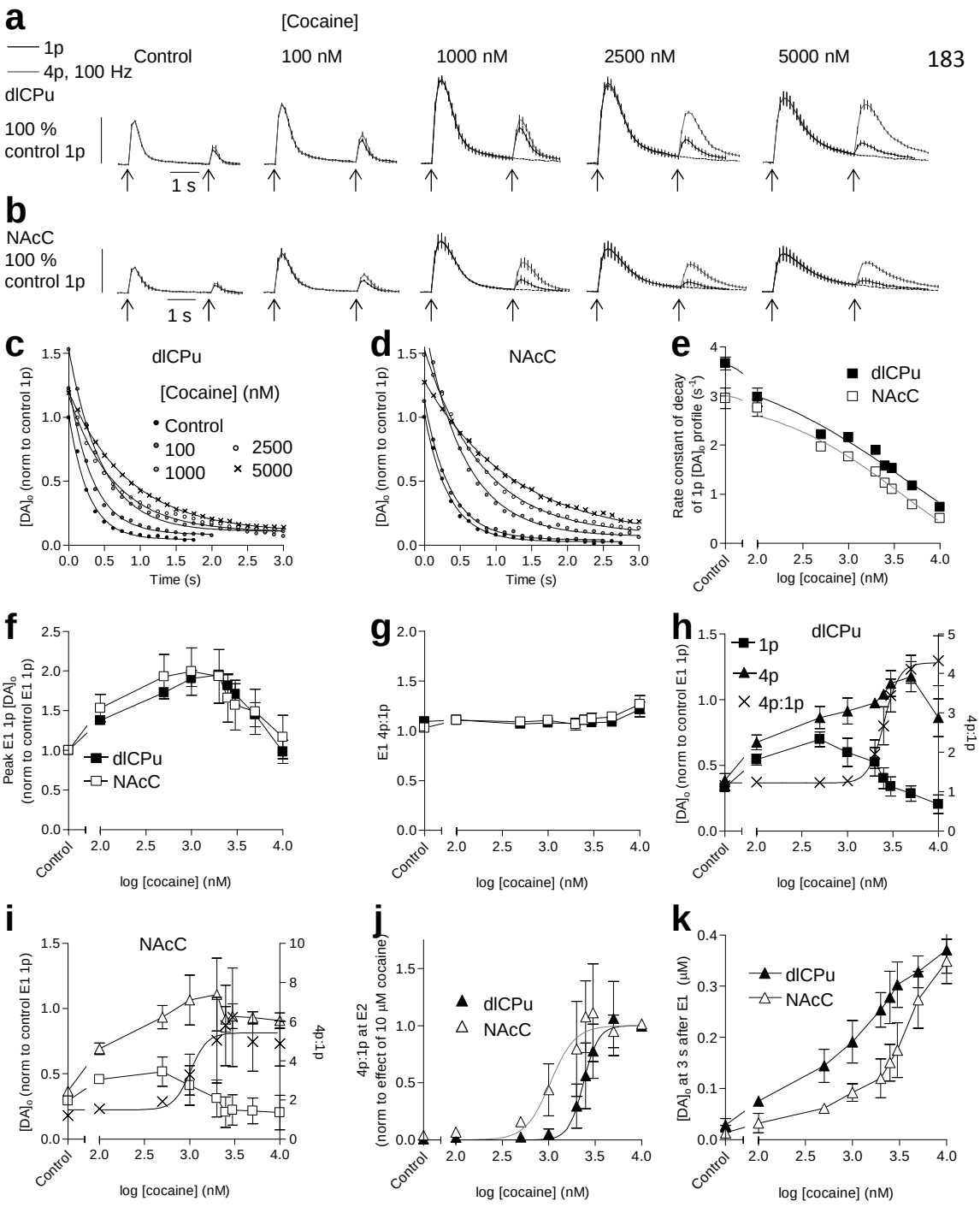

Figure 5.3. Cocaine promotes burst sensitivity of release at E2 with greater efficacy in NAcC vs. dlCPu (a,b) Profiles of mean $[DA]_o$±SEM normalised to peak $[DA]_o$ evoked by 1p in control conditions, against time, after an initial 1p stimulation (E1) followed 3 s later by 1p or 4p, 100 Hz stimulation (E2), with different cocaine concentrations (0-10 µM, limited concentrations shown), in dlCPu (a) and NAcC (b). **(c,d)** Decay phases of $[DA]_o$ profiles evoked by E1 1p, against time, in dlCPu (c) and NAcC (d) (limited cocaine concentrations shown). Curve fits are one phase exponential decays, $R^2>0.93$; $n = 3$. **(e)** Rate constant of decay of $[DA]_o$ profiles evoked by E1 1p, against log cocaine concentration. Curve fits are sigmoidal dose-response (variable slope) curves, $R^2>0.95$; $n = 3$. $LogEC_{50}$ is not different between dlCPu and NAcC ($p>0.05$, $F_{1,10}=3.46$; $n = 3$). **(f)** Peak $[DA]_o$±SEM evoked by E1 1p, against log cocaine concentration. **(g)** E1 4p:1p ±SEM against log cocaine concentration. **(h,i)** Mean peak $[DA]_o$±SEM evoked by E2 1p and 4p, normalised to peak $[DA]_o$ evoked by E1 1p in control conditions, and E2 4p:1p ±SEM, against log cocaine concentration, in dlCPu (h) and NAcC (i). Curve fits for 4p:1p are sigmoidal dose-response (variable slope) curves, dlCPu: $R^2=0.86$, NAcC: $R^2=0.40$, $n = 3$. **(j)** E2 4p:1p ±SEM, normalised to 4p:1p in 10 µM cocaine, against log cocaine concentration. Curve fits are sigmoidal dose-response (variable slope) curves, constrained to bottom = 0 and top = 1, $R^2>0.88$. $LogEC_{50}$ is significantly different between dlCPu and NAcC ($p<0.001$, $F_{1,14}=48.88$; $n = 3$). dlCPu: EC_{50}=2370 nM, NAcC: EC_{50}=1040 nM. **(k)** Mean $[DA]_o$±SEM at 3 s after E1 1p stimulation, against log cocaine concentration.

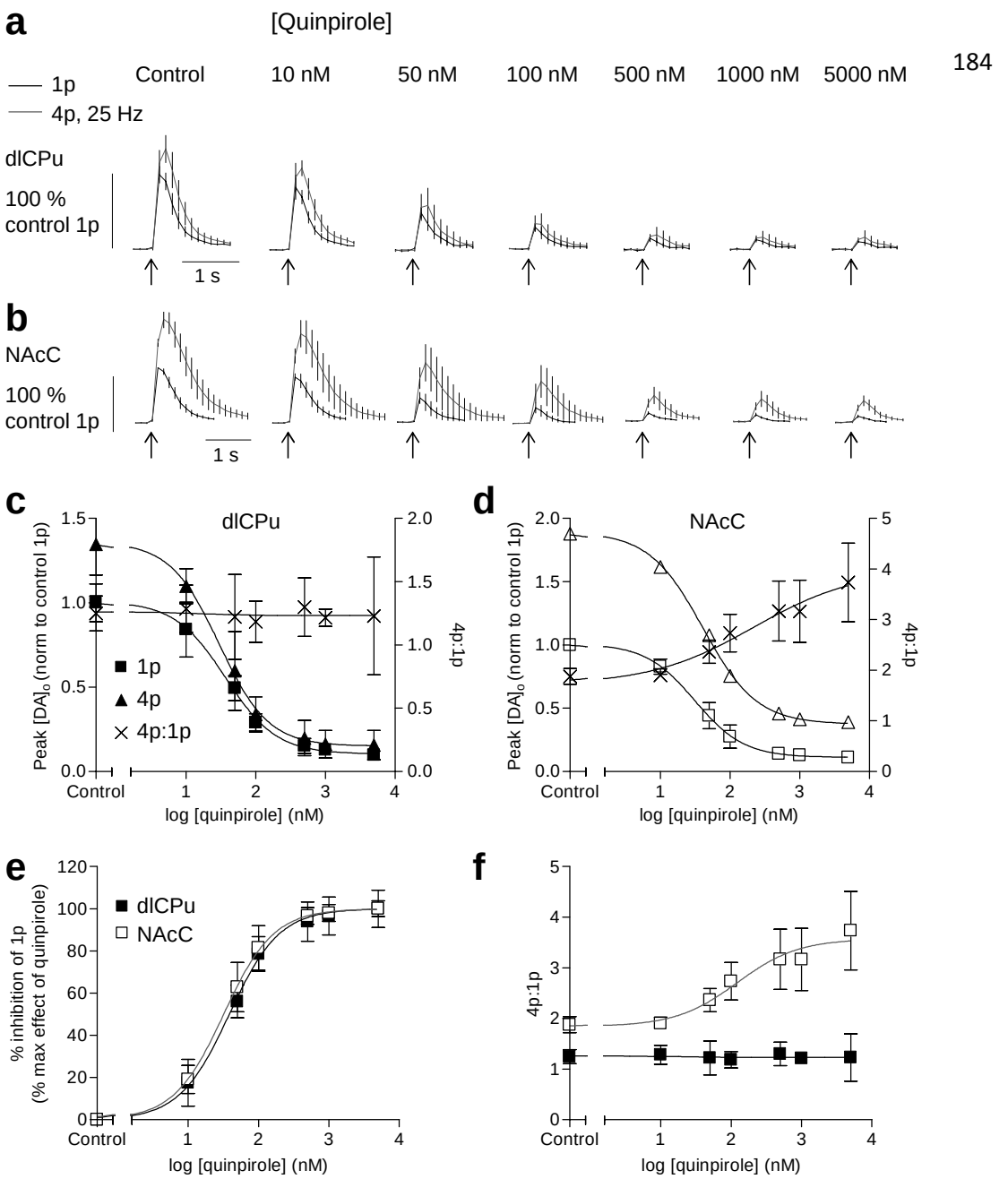

Figure 5.4. D_2R agonist dose-dependently controls DA release evoked by light-evoked DA neuron activity with slightly different efficacy in dlCPu vs. NAcC (a,b) Profiles of mean $[DA]_o$±SEM normalised to control peak $[DA]_o$ evoked by 1p against time, after single or burst light stimulation (arrow)(1p or 4p, 25 Hz), with different quinpirole concentrations (0-5 µM), in dlCPu (a) and NAcC (b) in $DAT^{Cre/+}$ mice transfected with AAV5-ChR2. **(c,d)** Mean peak $[DA]_o$±SEM evoked by 1p and 4p normalised to peak $[DA]_o$ evoked by 1p in control conditions, and mean 4p:1p ±SEM, against log quinpirole concentration, in dlCPu (c) and NAcC (d). Curve fits are sigmoidal dose-response (variable slope) curves. For peak $[DA]_o$ evoked by 1p and 4p, $R^2>0.76$; $n=3$. For 4p:1p, dlCPu: $R^2=0$, NAcC: $R^2=0.47$; $n=3$. **(e)** Percentage ±SEM of maximum effect of quinpirole on $[DA]_o$ evoked by 1p, against log quinpirole concentration. Curve fits are sigmoidal dose-response (variable slope) curves, constrained to 0 at bottom and 100 at top, $R^2>0.99$; $n=3$. $LogEC_{50}$ is significantly different between dlCPu and NAcC (dlCPu: EC_{50}=38.4 nM, NAcC: EC_{50}=32.0 nM $p<0.01$ $F_{1,11}$=13.28; $n=3$). **(f)** 4p:1p ±SEM against log quinpirole concentration. Curve fits are sigmoidal dose-response (variable slope) curves, dlCPu: $R^2=0$, NAcC: $R^2=0.47$; $n=3$.

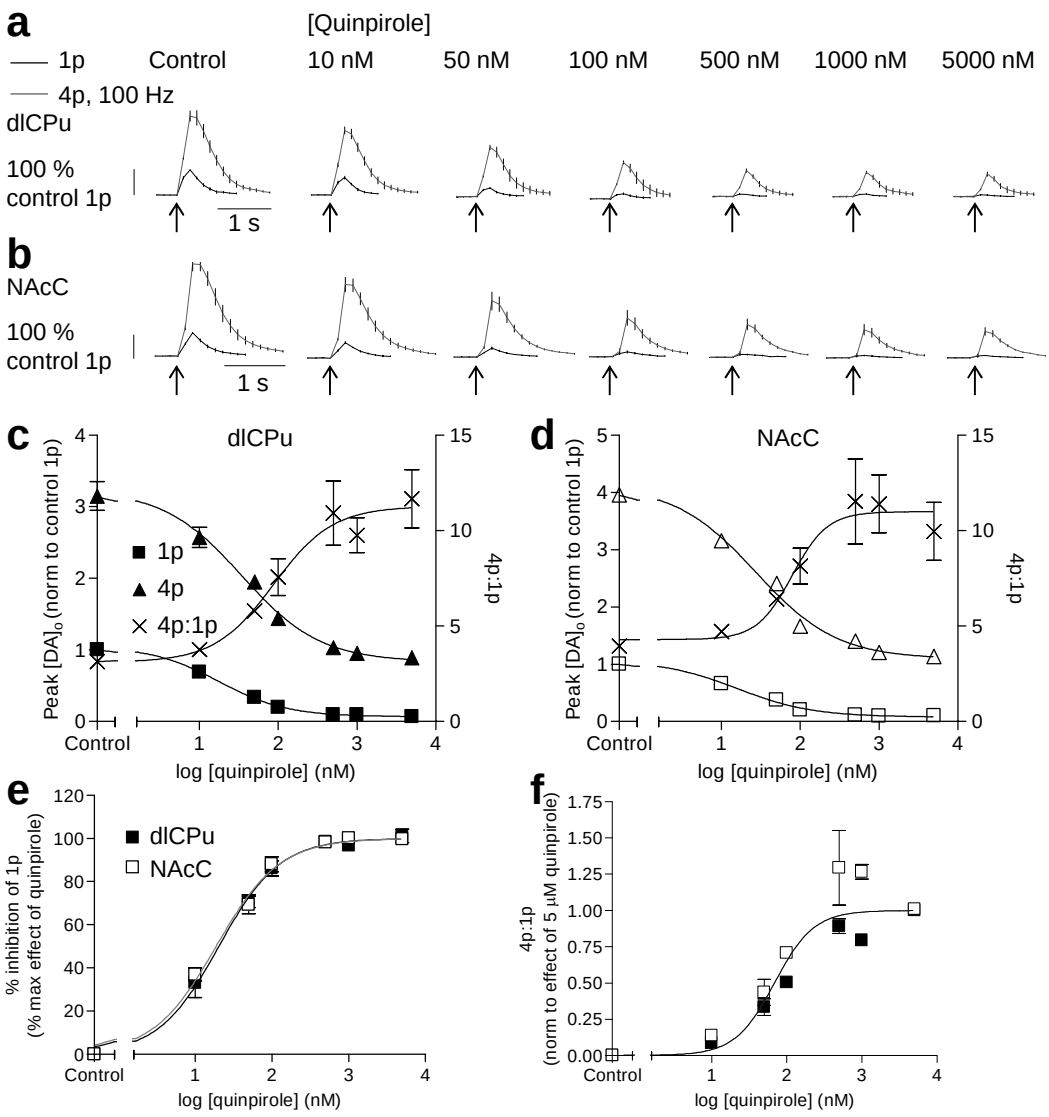

Figure 5.5. D_2R agonist dose-dependently controls electrically evoked DA release with nAChRs inhibited with similar efficacy in dlCPu and NAcC (a,b) Profiles of mean $[DA]_o$±SEM normalised to peak $[DA]_o$ evoked by 1p, against time, after 1p or 4p, 100Hz electrical stimulation (arrow), with DHβE (1 µM), with different quinpirole concentrations (0-5 µM), in dlCPu (a) and NACc (b). **(c,d)** Mean peak $[DA]_o$±SEM evoked by 1p and 4p normalised to peak $[DA]_o$ evoked by 1p in control conditions, and 4p:1p ±SEM, against log quinpirole concentration, in dlCPu (c) and NAcC (d). Curve fits are sigmoidal dose-response (variable slope) curves. For peak $[DA]_o$ evoked by 1p and 4p, $R^2>0.85$; $n=3$. For 4p:1p, $R^2>0.79$; $n=3$. **(e)** Percentage ±SEM of maximum effect of quinpirole on peak $[DA]_o$ evoked by 1p, against log quinpirole concentration. Curve fits are sigmoidal dose-response (variable slope) curves, constrained to 0 at bottom and 100 at top, $R^2>0.97$; $n=3$. $LogEC_{50}$ is not significantly different between dlCPu and NAcC ($p>0.05$, $F_{1,11}=0.39$; $n=3$. **(f)** 4p:1p ±SEM normalised to the effect of 5 µM quinpirole on 4p:1p, against log quinpirole concentration. Curve fits are sigmoidal dose-response (variable slope) curves constrained to 0 at bottom and 1.0 at top., $R^2>0.62$; $n=3$. $LogEC_{50}$ is not significantly different between dlCPu and NAcC ($p>0.05$, $F_{1,7}=0.41$; $n=3$).

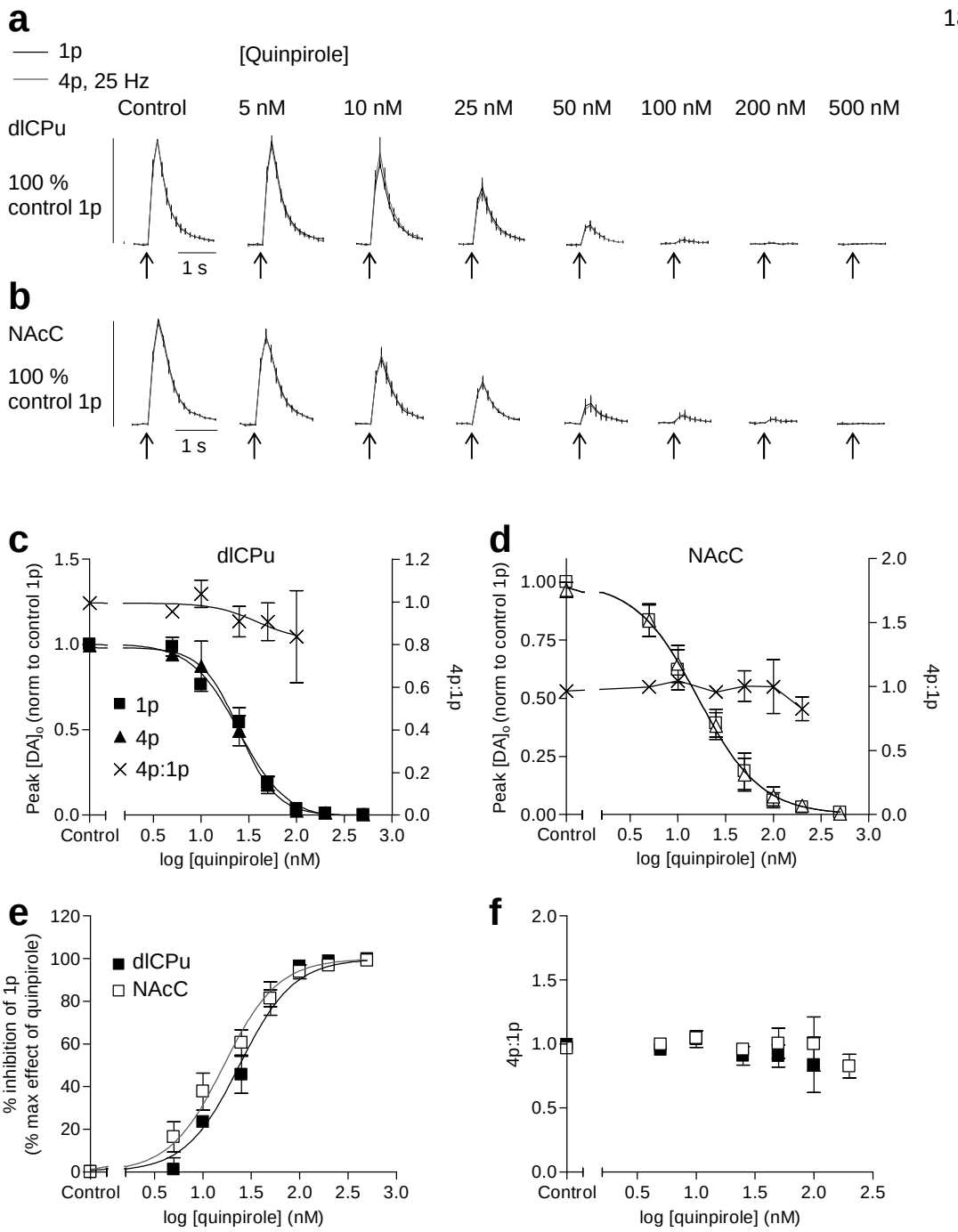

Figure 5.6. D_2R agonist dose-dependently controls DA release evoked by light-evoked ChI activity with greater efficacy in NAcC vs. dlCPu (a,b) Profiles of mean $[DA]_o$±SEM normalised to peak $[DA]_o$ evoked by 1p in control conditions, against time, after 1p or 4p, 25 Hz light stimulation (arrow), with different quinpirole concentrations (0-500 nM), in dlCPu (a) and NAcC (b) in $ChAT^{Cre/+}$ mice transfected with AAV5-ChR2. **(c,d)** Mean peak $[DA]_o$±SEM evoked by 1p and 4p normalised to peak $[DA]_o$ evoked by 1p in control conditions, and 4p:1p ±SEM, against log quinpirole concentration, in dlCPu (c) and NACc (d). Curve fits are sigmoidal dose-response (variable slope) curves. For peak $[DA]_o$ evoked by 1p and 4p, $R^2>0.95$; $n=3$. For 4p:1p, dlCPu: $R^2=0.12$, NAcC: does not converge, $n = 3$. 4p:1p not calculated for highest quinpirole concentrations where peak $[DA]_o$ evoked by 1p ≈0. **(e)** Percentage ±SEM of maximum effect of quinpirole on peak $[DA]_o$ evoked by 1p, against log quinpirole concentration. Curve fits are sigmoidal dose-response (variable slope) curves, constrained to 0 at bottom and 100 at top, $R^2>0.96$; $n=3$. $LogEC_{50}$ is significantly different between dlCPu and NAcC (dlCPu: EC_{50}=23.8 nM, NAcC: EC_{50}=16.4 nM $p<0.01$, $F_{1,13}$=10.97; $n=3$). **(f)** 4p:1p ±SEM against log quinpirole concentration. 4p:1p not calculated for highest quinpirole concentrations where peak $[DA]_o$ evoked by 1p ≈0.

6. A53Tα-syn subtly modifies striatal DA release

6.1 Introduction

In this chapter I have explored the role of one presynaptic protein, α-syn, in the control of striatal DA release, in a genetic mouse model of PD.

6.1.1 Parkinson's disease

PD is a progressive neurodegenerative disorder, with a prevalence of around 1% in over 60s (de Rijk *et al.* 2000). As described in 1.3.1, PD is usually considered to be primarily a motor disorder with cardinal symptoms of bradykinesia, rigidity and resting tremor, but can also include non-motor symptoms such as cognitive decline, depression, sleep disorders and autonomic dysfunction (Aarsland *et al.* 2008; O'Sullivan *et al.* 2008; Stacy 2011). The motor symptoms of PD are thought to be primarily due to the preferential degeneration of DA neurons in the ventral tier of the SNc (Fearnley and Lees 1991), and consequent loss of DA in the sensorimotor-associated dorsolateral striatum. According to the classic model of basal ganglia function, this reduction in sensorimotor striatal DA causes an imbalance in the motor-associated direct and indirect pathways of the basal ganglia, leading to deficits in motor function (Albin *et al.* 1989).

The mechanisms underlying DA neuron degeneration are not fully understood, and as described in 1.3.1, multiple interconnected cellular pathways have been implicated (Dawson and Dawson 2003; Bandopadhyay and de Belleroche 2010; Imai and Lu 2011). It has now been shown however that decreases in striatal DA levels and striatal DA terminal degeneration precede overt degeneration of DA neurons in the midbrain (Hornykiewicz 1998; Cheng *et al.* 2010). Therefore, one hypothesis is that presynaptic dysfunction and changes in striatal DA release are early events in PD pathogenesis. In this chapter I have investigated whether there are early changes in striatal DA release, prior to overt degeneration of DA neurons, in a genetic mouse model of PD.

6.1.2 α-synuclein

As discussed in chapter 1.3.1, the protein α-syn is strongly implicated in the pathogenesis of several familial forms of PD, as well as idiopathic PD. Multiplication of, or mutations in, *SNCA*, the gene coding for α-syn, cause dominantly inherited PD in humans (Polymeropoulos *et al.* 1997; Krüger *et al.* 1998; Singleton *et al.* 2003; Zarranz *et al.* 2004), and α-syn is the predominant component of Lewy bodies, the pathological protein aggregates which are hallmarks of PD (Spillantini *et al.* 1997). The physiological function of α-syn is incompletely understood. Most recent work has focussed on a role for α-syn in the control of synaptic vesicle dynamics, particularly vesicle fusion, trafficking and recycling (Abeliovich *et al.* 2000; Murphy *et al.* 2000; Cabin *et al.* 2002; Yavich *et al.* 2004; Larsen *et al.* 2006; Burré *et al.* 2010; Nemani *et al.* 2010; Anwar *et al.* 2011), although its precise role remains undefined. Reducing synuclein levels in cultured neurons or in mouse models alters vesicle dynamics and organisation (Murphy *et al.* 2000; Cabin *et al.* 2002; Anwar *et al.* 2011). However, synuclein knockout mice show increases in transmitter release (Senior *et al.* 2008; Anwar *et al.* 2011), suggestive of a role for α-syn as a negative regulator of transmitter release. Consistent with this, overexpressing α-syn causes deficits in transmitter release and vesicle cycling in cultured cells (Larsen *et al.* 2006; Nemani *et al.* 2010) and *in vivo* in response to prolonged stimulation (Yavich *et al.* 2005). However, the effect of mutant α-syn on striatal DA signalling, in regions differentially affected in PD, and in response to physiological stimulations, remains unexplored. Therefore here I have explored whether striatal DA release is disrupted in mice overexpressing mutant α-syn, which may have implications for understanding the early pathogenesis of PD.

6.1.3 A53Tα-syn overexpressors

Overexpression of mutant α-syn has been used to generate several mouse models of PD (Fernagut and Chesselet 2004; Dawson *et al.* 2010). Mouse lines overexpressing human A53Tα-syn under control of the mouse prion protein (PrP) promoter constitute one such model (Gispert

et al. 2003). These mice show progressive pathological changes in the absence of overt nigrostriatal degeneration, including abnormal subcellular localisation of α-syn in many brain regions, mild accumulation of insoluble α-syn in DA neurons in the SNc and non-specific signs of neurodegeneration in the olfactory bulb (Gispert *et al.* 2003). Furthermore, these mice show progressive presynaptic changes, including the upregulation of vesicle-associated proteins such as the chaperone 14-3-3 (Kurz *et al.* 2011), and progressive striatal postsynaptic changes, including increased DA receptor expression, altered expression of proteins downstream of DA signalling and reduced corticostriatal LTD (Kurz *et al.* 2010; Tozzi *et al.* 2011). They also show age-related motor deficits including reduced rearing, step length and rotarod performance (Gispert *et al.* 2003). These postsynaptic and behavioural changes are suggestive of progressive deficits in striatal DA signalling, but occur in the absence of nigrostriatal degeneration. Therefore, these mice may model early PD-associated presynaptic deficits and are a useful tool for exploring early changes in DA release.

In this chapter I have directly investigated whether A53Tα-syn overexpressing mice show deficits in striatal DA release. DA release has been explored in two lines of A53Tα-syn overexpressing mice, vs. WT mice, in dorsal and ventral striatal regions, which are differentially affected in PD. Mice were used at two age points, an adult age prior to the onset of motor impairments (6-14 months), and an age at which motor impairments have been reported (18-24 months).

6.2 Methods

6.2.1 Animals

Female mice overexpressing human A53Tα-syn under control of the mouse PrP promoter, described previously (Gispert *et al.* 2003), were compared to WTs of the same background strain, FVB/N. A53Tα-syn overexpressing mice and WT controls were kindly supplied by Prof Auburger and Dr Gispert-Sanchez (Goethe University Medical School, Germany). A53Tα-syn overexpressors

show an approximately 1.5 fold increase in α-syn protein in the brain vs. WT (Gispert *et al.* 2003; Kurz *et al.* 2010). Mice from two different founder lines (line A and line B) were used, to control for effects due to random gene insertion. Mice were used at two ages, an adult age, before the onset of motor impairments (6-14 months), and an age at which the onset of motor impairments has been reported (18-24 months) (Gispert *et al.* 2003). All procedures were carried out in accordance with UK Home Office project licence and local guidelines.

6.2.2 Slice preparation and FCV

Acute striatal slices were prepared and FCV carried out as described in chapter 2.

6.2.3 Electrical stimulation and experimental design

DA release was evoked using electrical stimulation, as described in chapter 2.3.1.

$[DA]_o$ can be highly variable between striatal recording sites. Therefore, in each animal $[DA]_o$ was measured in five subregions in CPu (dorsolateral, 1; dorsomedial, 2; ventrolateral, 3; ventromedial, 4; and central, 5;) and in two subregions in NAcC (lateral, 6; medial, 7)(**Fig. 6.1**), to obtain representative measurements of striatal DA release.

The following protocol consisting of three types of stimulation, (i), (ii) and (iii), separated by 2.5 minute intervals, was applied in each recording site:

(i) 1p, 1p (separated by 4 s)

(ii) 4p (100 Hz)

(iii) 1p

$[DA]_o$ evoked by the initial 1p of (i) was used as a measure of DA release in an unstimulated recording site. $[DA]_o$ evoked by 4p, 100 Hz, (ii), was used as a measure of DA release evoked by a burst of activity. This burst evoked $[DA]_{o,}$ (ii), was normalised to mean peak $[DA]_o$ evoked by the initial 1p of (i) and (iii). This is because, as described in 2.3.1.1, DA release *in vitro* usually declines over the initial 4-5 stimulations in a previously unstimulated recording site. $[DA]_o$ evoked by (iii), normalised to peak $[DA]_o$ evoked by the initial 1p of (i), was used as a measure of this decline. $[DA]_o$ evoked by the second 1p of (i), normalised to peak $[DA]_o$ evoked by the first 1p of (i), was used as a measure of recovery of DA release from short-term depression.

The frequency response of $[DA]_o$ was explored in a single recording site per animal, in dlCPu. Train stimulations (4p, 5-100 Hz), were applied in pseudorandomized order and in triplicate. The ratio of peak $[DA]_o$ evoked by 4p to that evoked by 1p (4p:1p) was determined for each frequency to explore the frequency sensitivity of DA release.

The recovery of DA release following a prolonged stimulation was explored in a single recording site per slice, in dlCPu. A prolonged stimulation of 600 pulses (10 Hz, 60 s) was applied and recovery of DA release monitored by 1p stimulations at 10, 30, 60, 120, 270 and subsequent 150 s intervals following prolonged stimulation. $[DA]_o$ was normalised in two ways. Firstly it was normalised to peak $[DA]_o$ evoked by the prolonged stimulation, to explore the extent of recovery. Secondly it was normalised to the maximum recovery of $[DA]_o$ following prolonged stimulation (defined as the mean peak $[DA]_o$ evoked by 1p stimulations 32.5-40 minutes after the end of prolonged stimulation), to explore the rate of recovery.

6.2.4 Data analysis

Data were acquired and analysed as described in chapter 2.2.2. Comparisons for means were carried out in GraphPad Prism using Unpaired t tests, One- or Two-way ANOVAs and *post hoc* Bonferroni's t tests. Curve fits were carried out in GraphPad Prism, using sigmoidal dose-response (variable slope), one phase exponential decay or two phase exponential association

analysis. *n* = number of recording sites, for experiments exploring $[DA]_o$ across the striatum (Cragg 2003; Anwar *et al.* 2011), since $[DA]_o$ shows as much variability between striatal subregions within animals as between animals. *n* = number of experiments, for experiments in a single recording site investigating frequency response and recovery of release following prolonged stimulation.

6.2.5 Drugs

D-(2*R*)-amino-5-phosphonopentanoate (D-AP5), bicuculline methiodide, DHβE, 4-(8-methyl-9*H*-1,3-dioxolo[4,5-*h*][2,3]benzodiazepin-5-yl)-benzenamine hydrochloride (GYKI 52466), L-714,626, (S)-α-methyl-4-carboxyphenylglycine (MCPG) and saclofen were purchased from Tocris Biosciences or Ascent Scientific. Drugs were dissolved in distilled water or aqueous alkali (MCPG), aqueous acid (GYKI 52466) or DMSO (L-741,626) to make stock aliquots 1000-10000x final concentrations and stored at -20^{o}C. Final concentrations were made up in aCSF immediately before use.

6.3 Results

6.3.1 A53Tα-syn overexpression does not affect $[DA]_o$ evoked by a single pulse

$[DA]_o$ was explored in two lines of A53Tα-syn overexpressing mice (lines A and B) vs. WT mice of the same background strain, at two age points, 6-14 months and 18-24 months. Firstly, $[DA]_o$ evoked by 1p was explored. $[DA]_o$ was measured in five subregions per animal in CPu and two subregions per animal in NAcC (**Fig. 6.1**). Data was then averaged within CPu and NAcC. Consistent with previous studies(Cragg 2003; Anwar *et al.* 2011), peak $[DA]_o$ evoked by 1p was lower in NAcC than in CPu in all genotypes, at both ages (**Fig. 6.2 a,b**). However, there was no difference between genotypes in peak $[DA]_o$ evoked by 1p, at either age, in either CPu or NAcC (One-way ANOVA, $p > 0.05$; *n = 15-40* (CPu), *n = 6-14* (NAcC))(**Fig. 6.2 a,b**). There was also no significant difference in peak $[DA]_o$ evoked by 1p between 6-14 month vs. 18-24 month mice for

any genotype, in either region (Unpaired t tests, $p > 0.05$; $n = 6\text{-}40$) (**Fig. 6.2 a,b**). Cumulative distribution plots of peak 1p $[DA]_o$ showed similar spreads of data in all genotypes in CPu at 6-14 months and in NAcC at both ages, since sigmoidal curve fits were not different between genotypes ($p > 0.05$; $R^2 > 0.93$) (**Fig. 6.2 c**). However, in CPu in 18-24 month mice, sigmoidal curve fits of cumulative distribution plots were significantly different between genotypes ($p < 0.05$, $R^2 > 0.98$) (**Fig. 6.2 c**). There was a slightly reduced slope in A53Tα-syn overexpressors, suggesting a small relative increase in low-releasing sites, or decrease in high-releasing sites.

The falling phases of DA profiles, which are a function of DA uptake (Giros *et al.* 1996), were compared between genotypes to explore any changes in DA uptake. Since uptake is concentration dependent, profiles were concentration matched. All profiles with peak 1p $[DA]_o$ greater than 1.5 µM in CPu and 0.8 µM in NAcC, were synchronised to the time point at which $[DA]_o$ fell below these concentrations, and the falling phases averaged within region. These concentrations were selected to balance providing a sufficiently large falling phase for analysis and including enough profiles from each genotype for a valid comparison. Falling phases were curve fit using a one phase exponential decay ($R^2>0.96$). Curve fits were not significantly different between genotypes in either region at either age ($p > 0.05$; $n = 4\text{-}28$) (**Fig. 6.3**).

Therefore A53Tα-syn overexpression does not grossly affect $[DA]_o$ evoked by 1p in either CPu or NAcC, although there may be subtle differences in the spread of release sites between genotypes, in CPu, in aged mice.

6.3.2 A53Tα-syn overexpression does not affect $[DA]_o$ evoked by burst stimulation

As discussed previously, DA neurons show two main firing patterns *in vivo*: low frequency tonic firing (2-5 Hz) and short bursts of high frequency firing (2-4 pulses, 5-40 Hz or higher) (Grace and Bunney 1984; Hyland *et al.* 2002), which have different behavioural significance (Schultz 2007). α-syn has been proposed to be involved in some forms of the short-term plasticity of DA release

(Abeliovich *et al.* 2000). Therefore the dynamic filtering of these firing patterns into striatal DA release may potentially involve α-syn. Hence $[DA]_o$ evoked by high frequency burst stimulation (4p, 100 Hz), was also explored, in CPu and NAcC at both ages. Again, five subregions in CPu and two subregions in NAcC per animal were used, and data averaged within region. There was no significant difference between genotypes in peak $[DA]_o$ evoked by burst stimulation, normalised to 1p release, in either region, at either age (One-way ANOVA, $p > 0.05$; CPu: $n = 15\text{-}40$, NAcC: $n = 6\text{-}14$) (**Fig. 6.4 a,b**). Therefore, A53Tα-syn overexpression does not affect DA release in response to burst stimulation, in drug-free conditions.

6.3.3. A53Tα-syn overexpression does not affect the frequency sensitivity of $[DA]_o$

The filtering of different firing patterns into striatal DA release is highly dynamic and, as discussed in previous chapters, depends on multiple presynaptic processes acting on multiple timescales. It is possible that A53Tα-syn overexpression may affect presynaptic processes on any of these timescales. Therefore, in addition to investigating $[DA]_o$ evoked by high frequency bursts as described above, $[DA]_o$ evoked by stimulations across a full range of frequencies (4p, 5-100 Hz) was investigated, in a single subregion, dlCPu, at both ages (**Fig. 6.5 a,b upper**). The ratio of peak $[DA]_o$ evoked by 4p vs. 1p (4p:1p) was calculated at each frequency.

The frequency response of $[DA]_o$ formed a characteristic inverted-U shape in all genotypes at both ages (**Fig. 6.5 c**). This shape is due to a number of processes, including short-term depression of release, D_2 receptor-dependent suppression of release at low frequencies and the frequency-dependent extracellular summation of DA (Cragg 2003; Rice and Cragg 2004). There was no significant interaction between genotype and frequency at either age (**Fig. 6.5 c**) (Two-way ANOVA, $p > 0.05$; $n = 3\text{-}5$), although at 18-24 months there was a main effect of genotype (Two-way ANOVA, $p < 0.01$; $n = 3\text{-}5$), probably due the slightly increased 4p:1p at 10 Hz stimulation, in line B A53Tα-syn overexpressors (**Fig. 6.5 c right**). These results suggest that

A53Tα-syn overexpression has little effect on the short-term plasticity of DA release, but may cause subtle changes in aged animals. However, since this difference is only seen in one line and this frequency response is dependent on multiple factors, these results are hard to interpret.

As described previously, one strong determinant of the frequency sensitivity of $[DA]_o$ is ACh released from ChIs, acting at presynaptic nAChRs (Rice and Cragg 2004; Zhang and Sulzer 2004). Therefore, to explore the effects of A53Tα-syn overexpression on the frequency sensitivity of DA release in the absence of the confounding effects of ACh, $[DA]_o$ evoked by stimulations of different frequencies (4p, 5-100 Hz) was explored with nAChRs inhibited, (DHβE, 1 μM) (**Fig. 6.5 a,b lower**). With DHβE, 4p:1p was strongly dependent on frequency, increasing with increasing frequency, as previously reported (Rice and Cragg 2004), in all genotypes at both ages (**Fig. 6.5 d**). There was no effect of genotype on this frequency response, at either age (Two-way ANOVA, *p > 0.05*; *n = 3-5*), although there was a slight trend towards decreased 4p:1p at high frequencies $[DA]_o$, in A53Tα-syn overexpressors at 18-24 months. This suggests that A53Tα-syn overexpression does not greatly modulate the frequency sensitivity of DA release, when the confounding effects of ACh are inhibited.

6.3.4 A53Tα-syn overexpression does not affect subsequent DA release following modest stimulation

As described in 2.3.1, DA release *in vitro* declines over the initial 4-5 1p stimulations, separated by 2.5 minute intervals, applied to an unstimulated recording site, before release becomes sustainable and reproducible for several hours. In addition, as discussed previously, DA release *in vitro* shows strong short-term depression (Kennedy *et al.* 1992; Abeliovich *et al.* 2000), recovering to reproducible levels over a time course of around 60-90 s. The presynaptic processes underlying this initial decline in DA release in unstimulated recording sites and the short-term depression of DA release are not well understood. However it is possible that they involve changes in the availability, trafficking or release of synaptic DA vesicles. α-syn has been

implicated in the control of vesicle trafficking (Murphy *et al.* 2000; Cabin *et al.* 2002; Nemani *et al.* 2010) and in the recovery of DA release from short-term depression (Abeliovich *et al.* 2000). Therefore the effect of A53Tα-syn overexpression on DA release following prior stimulation, over both a timescale of minutes in previously unstimulated recording sites, and a timescale of seconds, has been investigated.

Firstly, the effect of A53Tα-syn overexpression on $[DA]_o$ evoked by 1p, 5 minutes after the initial stimulation in a previously unstimulated site and 2.5 minutes after the previous stimulation at that site (i.e. $[DA]_o$ evoked by stimulation (iii), see methods) was explored (**Fig. 6.6 a**). There was no difference between genotypes in peak $[DA]_o$ evoked by this stimulation, normalised to initial 1p $[DA]_o$, at either age, in either region (One-way ANOVA, *p>0.05*; *n=15-40* (CPu), *n=6-14* (NAcC)) (**Fig. 6.6 a,b**).

Secondly, the effect of A53Tα-syn overexpression on $[DA]_o$ evoked by 1p, 4 s after an initial 1p stimulation, was explored, to investigate recovery of DA release from short-term depression (**Fig. 6.7 a**). There was no difference between genotypes in peak $[DA]_o$ evoked by this stimulation, normalised to initial 1p $[DA]_o$, at either age, in either region (One-way ANOVA, *p>0.05*; *n=15-40* (CPu), *n=6-14* (NAcC)) (**Fig. 6.7 a,b**).

Therefore A53Tα-syn overexpression has no effect on the depression of DA release on a timescale of either minutes or seconds, following single pulse stimulation.

6.3.5 A53Tα-syn overexpression increases recovery of DA release following prolonged stimulation

It has been reported that α-syn may control vesicle recycling or reclustering following prolonged stimulation (Yavich *et al.* 2004; Nemani *et al.* 2010). Therefore the effect of A53Tα-syn overexpression on recovery of DA release following prolonged stimulation was also explored. This was investigated in dlCPu, in 18-24 month mice only. These experiments were carried out in

the presence of a mixture of antagonists for glutamate (GYKI52466, 10 µM; D-AP5, 50 µM; MCPG, 200 µM), GABA (bicuculline, 10 µM; saclofen, 50 µM) and D_2 (L-741,626, 1 µM) receptors. This is because, whilst glutamate and GABA transmission do not affect DA release evoked by short discrete stimuli (Cragg 2003; Zhang and Sulzer 2003), they may play a role in controlling DA release during more prolonged stimulations (Zhang and Sulzer, 2003), and D_2Rs may also be activated over this time course (Benoit-Marand *et al.* 2001; Phillips *et al.* 2002). Therefore, to ensure any differences between genotypes were due to presynaptic effects of A53Tα-syn overexpression in DA terminals and not other network effects, the cocktail of antagonists was applied throughout. In a single recording site per slice, in dlCPu, a prolonged stimulation was applied (10 Hz, 60 s). Recovery of $[DA]_o$ evoked by 1p stimulation was monitored at 10, 30, 60, 120, 270 and subsequent 150 s intervals following the end of prolonged stimulation.

There was no difference between genotypes in peak $[DA]_o$ evoked by the prolonged stimulation, normalised to prior 1p release (One-way ANOVA, $p > 0.05$; $n = 9$) (**Fig. 6.8 a**). Following prolonged stimulation, $[DA]_o$ evoked by 1p recovered to 20-40% of peak $[DA]_o$ evoked by 1p prior to prolonged stimulation, over a time course of 20-40 mins, in all genotypes. To investigate both the extent and rate of recovery, data was normalised both to peak $[DA]_o$ evoked by the prolonged stimulation (**Fig. 6.8 b**), and to maximum recovery of $[DA]_o$ (**Fig. 6.8 c**). Data were curve fit using two phase exponential associations, $R^2>0.80$, and both the extent (**Fig. 6.8 b**) and rate (**Fig. 6.8 c**) of recovery were significantly different between genotypes ($p < 0.05$; $n = 8$-10). A53Tα-syn overexpressors showed both an increased extent and rate of recovery of DA release following prolonged stimulation compared to WT.

6.4 Discussion

The A53T mutation in *SNCA*, the gene coding for α-syn, causes dominant familial PD in humans (Polymeropoulos *et al.* 1997), and mouse lines overexpressing A53Tα-syn may model the early

stages of PD (Gispert *et al.* 2003; Kurz *et al.* 2010; Kurz *et al.* 2011). These mouse lines show progressive striatal postsynaptic changes suggestive of deficits in striatal DA signalling (Kurz *et al.* 2010; Tozzi *et al.* 2011), and changes in presynaptic protein expression which may indicate presynaptic deficits (Kurz *et al.* 2011). However, the effect of mutant α-syn on striatal DA signalling, in response to physiological stimuli, has not been directly explored. Therefore, in this chapter I explored whether overexpression of A53Tα-syn causes early deficits in striatal DA release. Whilst DA release is grossly unchanged in A53Tα-syn overexpressors in response to physiological stimuli, they show subtle changes in DA handling, with increased recovery of release following prolonged stimulation. These results suggest that, while the effects are subtle, mutant α-syn can modify the presynaptic release of striatal DA in the absence of overt nigrostriatal degeneration, which may contribute to the postsynaptic and behavioural deficits found in this mouse model of PD.

6.4.1 Absolute $[DA]_o$ is unchanged by A53Tα-syn overexpression

It has been proposed that synuclein functions as a negative regulator of neurotransmitter release in both dopaminergic and non-dopaminergic systems. Synuclein knockout animals show enhanced striatal DA signalling (Senior *et al.* 2008; Anwar *et al.* 2011), and conversely overexpression of α-syn reduces transmitter release in cultured cells (Larsen *et al.* 2006; Nemani *et al.* 2010). In addition, synucleins have been shown to differentially regulate DA release in CPu and NAcC (Anwar *et al.* 2011), which are differentially affected in PD. Therefore I first explored whether A53Tα-syn overexpression impaired striatal DA release in these two striatal regions. There was no difference in peak $[DA]_o$ evoked by a single stimulation in A53Tα-syn overexpressors vs. WT animals, in either CPu or NAcC, at either an adult age (6-14 months) or in aged mice (18-24 months). This suggests that A53Tα-syn overexpression does not grossly alter striatal DA release. However, these mice are reported to show a 20-30% increase in striatal DA

content compared to WTs (Kurz *et al.* 2010), so similar levels of evoked DA release compared to WTs may indicate a slight decrease in DA releasability.

The cumulative distribution of individual release sites was also largely similar between genotypes, suggesting that no subset of DA release sites was affected by A53Tα-syn overexpression. The slope of the cumulative distribution plot was slightly decreased in the CPu of aged A53Tα-syn overexpressors, which may suggest a slight relative decrease in low- or increase in high-releasing sites.

6.4.2 DA uptake is unchanged by A53Tα-syn overexpression

In addition to its role in controlling transmitter release, α-syn is known to form protein-protein interactions with the DAT (Lee *et al.* 2001; Wersinger and Sidhu 2003) and both WT and mutant α-syn have been reported to alter surface expression of the DAT, and hence DA uptake (Sidhu *et al.* 2004; Fountaine *et al.* 2008; Graham and Sidhu 2010). Therefore DA uptake in A53Tα-syn overexpressing mice was also explored. DA profiles were concentration matched, since uptake is dependent on extracellular concentration. The falling phases of concentration matched profiles, which are dependent on DA uptake (Giros *et al.* 1996), were not different between genotypes, suggesting that A53Tα-syn overexpression does not modulate DA uptake.

6.4.3 Short-term plasticity of DA release is unchanged by A53Tα-syn overexpression

DA neurons *in vivo* show two main firing patterns: low frequency tonic firing and high frequency burst firing, with differing behavioural relevance (Schultz 2007). These firing patterns are dynamically filtered into DA release, in a manner that depends upon multiple presynaptic and striatal network factors, as has been explored in the rest of this thesis. α-syn may be one presynaptic protein involved in controlling this dynamic filtering, since α-syn has been implicated in DA vesicle trafficking and hence the plasticity of DA release (Abeliovich *et al.* 2000; Cabin *et al.*

2002; Yavich *et al.* 2005; Nemani *et al.* 2010). Therefore, in addition to single pulse release, I have explored DA release evoked by train stimuli across the relevant range of DA neuron firing frequencies. Firstly, DA release evoked by high frequency burst stimuli was explored in drug-free conditions, across sampling sites in both CPu and NAcC. There was no difference between genotypes in normalised $[DA]_o$ evoked by burst stimuli, in either region, in either adult or aged mice, suggesting that, similarly to single pulse release, burst release is not affected by A53Tα-syn overexpression.

However, as has been explored throughout this thesis, the short term plasticity of DA release strongly depends on stimulation frequency and striatal network factors, particularly the actions of ACh at presynaptic nAChRs. Secondly therefore, the full frequency response of DA release was explored in dlCPu, both in drug-free conditions and when the confounding effects of ACh were inhibited using a nAChR antagonist. In drug-free conditions, the frequency response of DA release of all genotypes formed an inverted-U shape, consistent with previous reports (Rice and Cragg 2004; Exley *et al.* 2007; Exley and Cragg 2008). This frequency response was largely similar between genotypes, although there was a slight increase in relative DA release at intermediate frequencies in one line of aged A53Tα-syn overexpressors. This effect is difficult to interpret since it was only seen in one line, at one age, and this frequency response is a compound effect, dependent on presynaptic and network factors. Therefore the frequency response of DA release when the confounding effects of ACh were inhibited using a nAChR antagonist was also investigated. When nAChRs were inhibited, all genotypes showed a classic strong frequency dependence of DA release (Rice and Cragg 2004), and there was no significant difference in frequency response between genotypes, although there was a slight trend towards decreased burst sensitivity at high frequencies in aged A53Tα-syn overexpressors. These results suggest that A53Tα-syn overexpression does not strongly modulate the frequency response of DA release when the confounding effects of ACh are inhibited. Together these data suggest that mutant α-syn does not greatly modulate the short-term plasticity of DA release.

6.4.4 DA release after modest stimulation is unchanged by A53Tα-syn overexpression

In vitro, DA synapses show several forms of depression of release, including an initial decline in release over the first few stimulations in a previously unstimulated recording site and strong short-term depression of release following single pulse stimulation, which recovers over a time course of 30-60 seconds (Kennedy *et al.* 1992; Abeliovich *et al.* 2000). The presynaptic mechanisms underlying this plasticity are unknown, but may involve changes in the availability of synaptic vesicles. α-syn has been implicated in the control of vesicle pool size and trafficking, and hence in the control of short-term depression of release (Abeliovich *et al.* 2000; Murphy *et al.* 2000; Cabin *et al.* 2002). Additionally, A53Tα-syn overexpressors show upregulation of several proteins involved in vesicle cycling and trafficking including the chaperone protein 14-3-3 and the endocytic protein dynamin (Kurz *et al.* 2011). Therefore the effect of A53Tα-syn overexpression on these two forms of depression of DA release was investigated. A53Tα-syn overexpression had no effect on either initial decline of release in a previously unstimulated recording site over a time course of minutes, or on short-term depression of release over a time course of seconds, suggesting that A53Tα-syn does not affect vesicle trafficking or short-term depression of release following modest stimulation.

6.4.5 Changes in DA release following prolonged stimulation indicate changes in synaptic function

It has been reported that α-syn can affect vesicle reclustering following prolonged stimulation (Nemani *et al.* 2010). Therefore to probe whether there are subtle changes in synaptic function and vesicle trafficking in A53Tα-syn overexpressors, release of DA following an unphysiological prolonged stimulation was also explored. A53Tα-syn overexpressors showed an increased rate and extent of recovery of release following prolonged stimulation. This suggests that A53Tα-syn overexpression does cause subtle changes in vesicle handling in DA terminals, which become apparent under extreme conditions. The mechanisms controlling vesicle trafficking or vesicle

pools in DA terminals are not well understood. It is possible that this enhanced recovery of release reflects an increase in the size of a reserve pool of vesicles in A53Tα-syn overexpressors, which would be consistent with the decrease in reserve pool reported in hippocampal cells from α-syn knockout mice (Cabin *et al.* 2002) and also with the increased DA content reported in A53Tα-syn overexpressors (Kurz *et al.* 2010). Alternatively this enhanced recovery may reflect the increase in the vesicle chaperone protein 14-3-3 reported in these mice (Kurz *et al.* 2011), which may increase availability of vesicles for release. This increase in recovery is inconsistent however with the deficits in vesicle reclustering reported in cultured neurons overexpressing α-syn (Nemani *et al.* 2010). It is possible that the enhanced recovery described here reflects compensatory mechanisms found *in vivo*, but not in cultured cells, to offset subtle changes in DA releasability.

6.5. Implications and summary

In this chapter I have explored how mutant α-syn can affect presynaptic DA release and release plasticity in a mouse model of early PD. DA transmission was largely unchanged in these mice, but they showed subtle changes including an enhanced recovery of DA release following prolonged stimulation, and maybe a decrease in DA releasability since DA content is increased in these animals but DA release was unchanged. These changes are more subtle than the reported effects of mutant α-syn overexpression in cultured cells (Nemani *et al.* 2010), which may reflect compensatory mechanisms *in vivo* to normalise DA signalling. However, these subtle changes suggest that mutant α-syn causes small changes in DA presynaptic function, which *in vivo* over the lifetime of the animal, with continuous DA neuron activity and DA release, may contribute to the postsynaptic and motor deficits reported in these mice. These results support the hypothesis that subtle presynaptic changes may occur in nigrostriatal DA neurons early in PD, before DA

neurons in the midbrain begin to die and suggest that α-syn is one presynaptic protein that plays a subtle role in the control of striatal DA release and release plasticity.

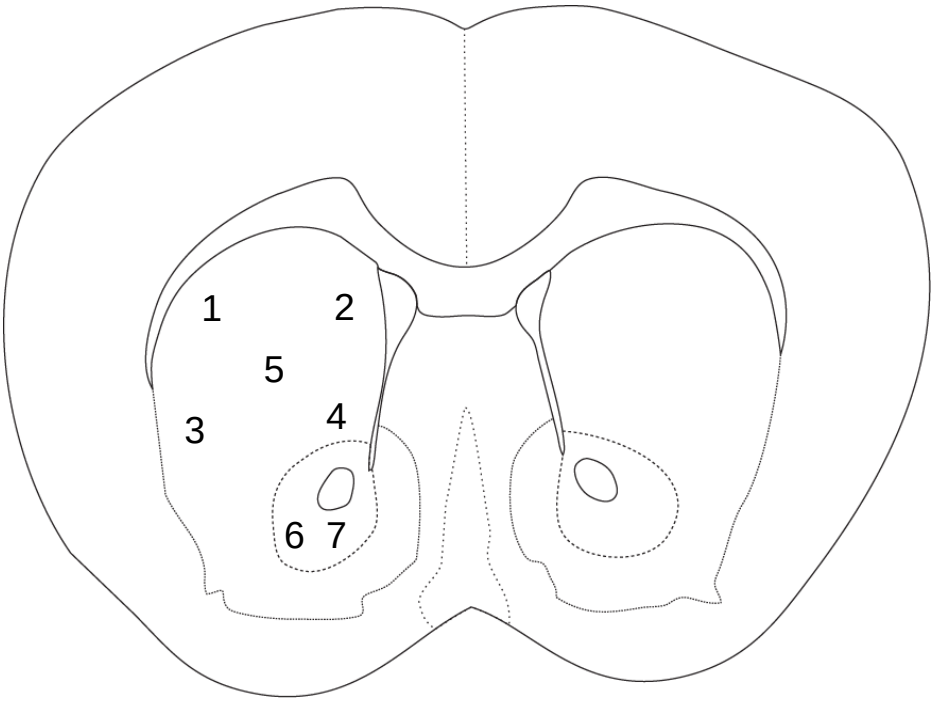

Figure 6.1. Diagram of coronal brain slice with locations of recording sites numbered. To obtain representative measurements of striatal DA release, $[DA]_o$ was monitored at 5 sites in CPu: 1 - dorsolateral, 2 - dorsomedial, 3 - ventrolateral, 4 - ventromedial, 5 - central, and 2 sites in NAcC: 6 - lateral, 7 - medial.

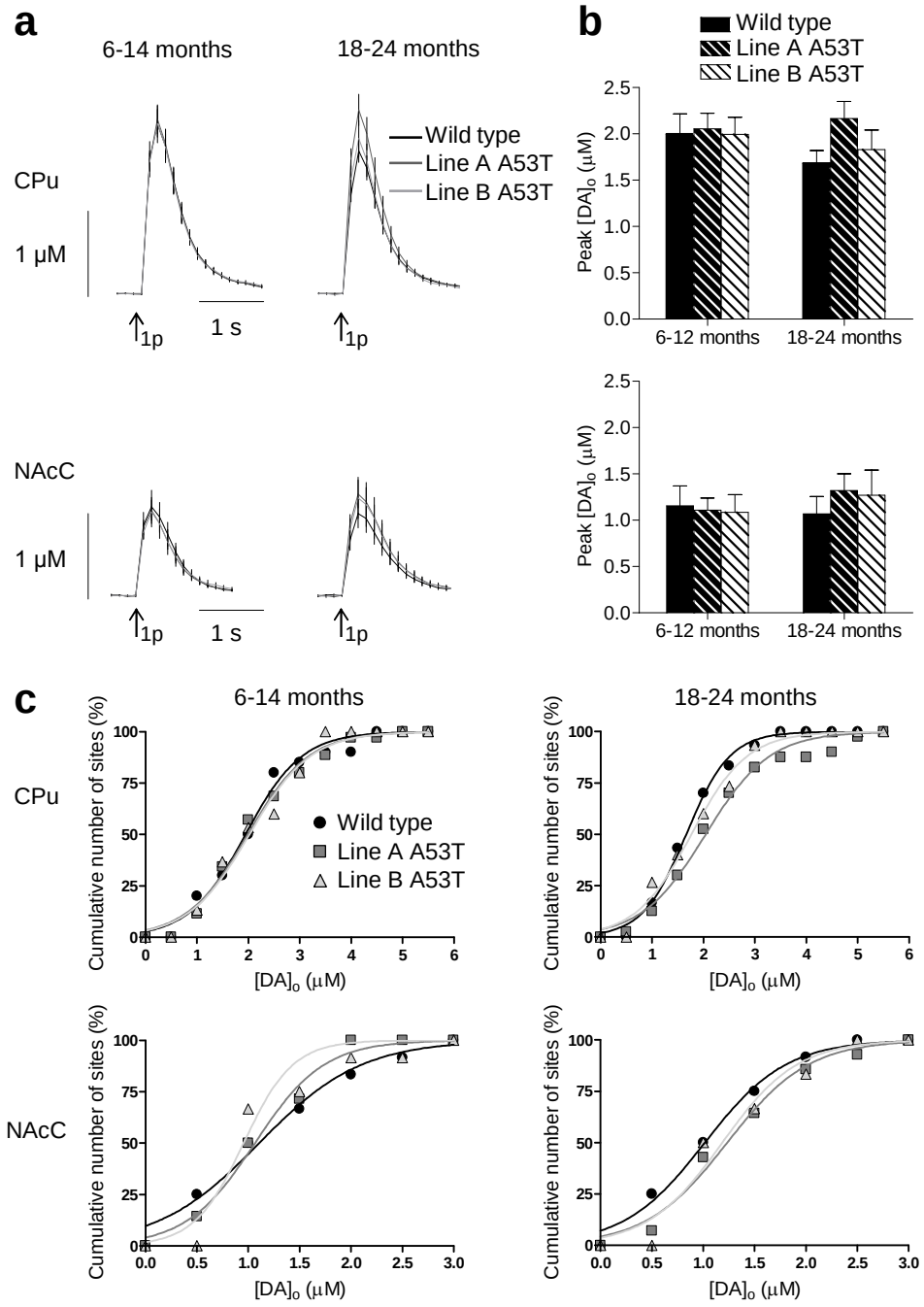

Figure 6.2. A53Tα-syn overexpression does not affect peak $[DA]_o$ evoked by a single pulse stimulation. **(a)** Profiles of mean $[DA]_o$±SEM against time after 1p stimulation (arrow). There was no significant difference in peak $[DA]_o$ between genotypes, at either age or in either region (One-way ANOVA, CPu 6-14 months *p>0.05*, $F_{2,54}$=0.00, CPu 18-24 months *p>0.05*, $F_{2,109}$=0.02; *n=15-40*; NAcC 6-14 months *p>0.05*, $F_{2,45}$=0.24, NAcC 18-24 months *p>0.05*, $F_{2,57}$=0.16; *n=6-14*). **(b)** Mean peak $[DA]_o$±SEM evoked by 1p. **(c)** Cumulative distribution plots of peak $[DA]_o$ evoked by 1p. Curve fits are sigmoidal variable slope curves, $R^2 > 0.93$. Curves are not significantly different between genotypes at 6-14 months in CPu or at either age in NAcC (CPu 6-14 months *p>0.05*, $F_{4,30}$=0.27, NAcC 6-14 months *p>0.05*, $F_{4,15}$=1.32, NAcC 18-24 months *p>0.05*, $F_{4,15}$=0.28) but are significantly different in CPu at 18-24 months (CPu 18-24 months *p<0.05*, $F_{4,30}$=8.32).

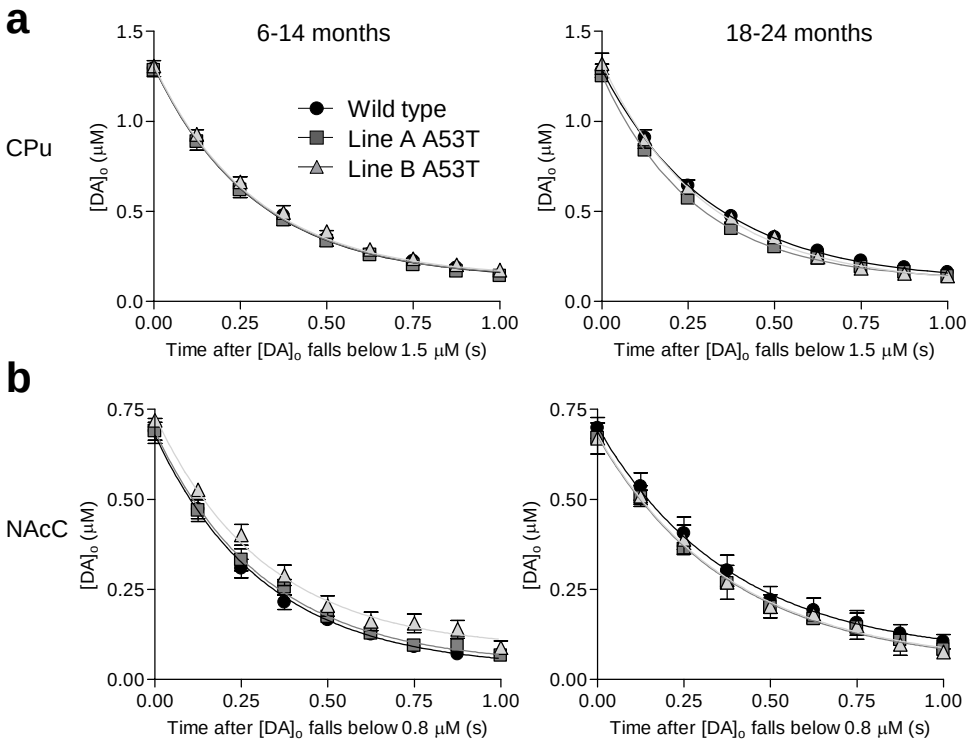

Figure 6.3. A53Tα-syn overexpression does no affect DA uptake. (a,b) Mean decay phases of concentration-matched DA profiles in CPu (a) and NacC (b). Curve fits are one phase exponential decays, R^2*>0.96*. Curves are not significantly different in either region at either age (Cpu: 6-14 months *p > 0.05*, $F_{4,117}$=0.08; 18-24 months *p > 0.05*, $F_{6,123}$=0.22; *n = 9-28*, NAcC: 6-14 months *p > 0.05*, $F_{4,18}$=1.54; 18-24 months *p > 0.05*, $F_{4,18}$=0.81; *n = 4-11*).

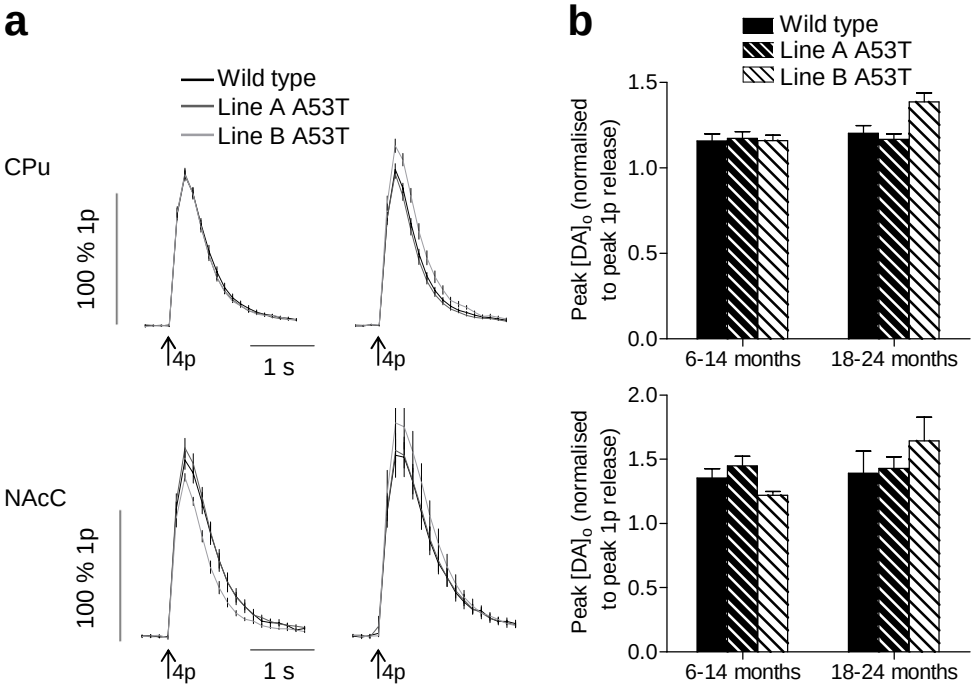

Figure 6.4. A53Tα-syn overexpression does not affect $[DA]_o$ evoked by burst stimulation. (a) Profiles of mean $[DA]_o$±SEM evoked by burst stimulation (4p, 100Hz, arrow) normalised to peak $[DA]_o$ evoked by 1p, against time after stimulation. There was no significant difference in peak $[DA]_o$ between genotypes, at either age or in either region (One-way ANOVA, CPu: 6-14 months *p>0.05*, $F_{2,96}$=0.00; 18-24 months *p>0.05*, $F_{2,93}$=0.26; *n=15-40*, NAcC: 6-14 months *p>0.05*, $F_{2,57}$=0.32; 18-24 months *p>0.05*, $F_{2,57}$=0.15; *n=6-14*). **(b)** Mean peak $[DA]_o$±SEM evoked by burst stimulation, normalised to peak $[DA]_o$ evoked by 1p.

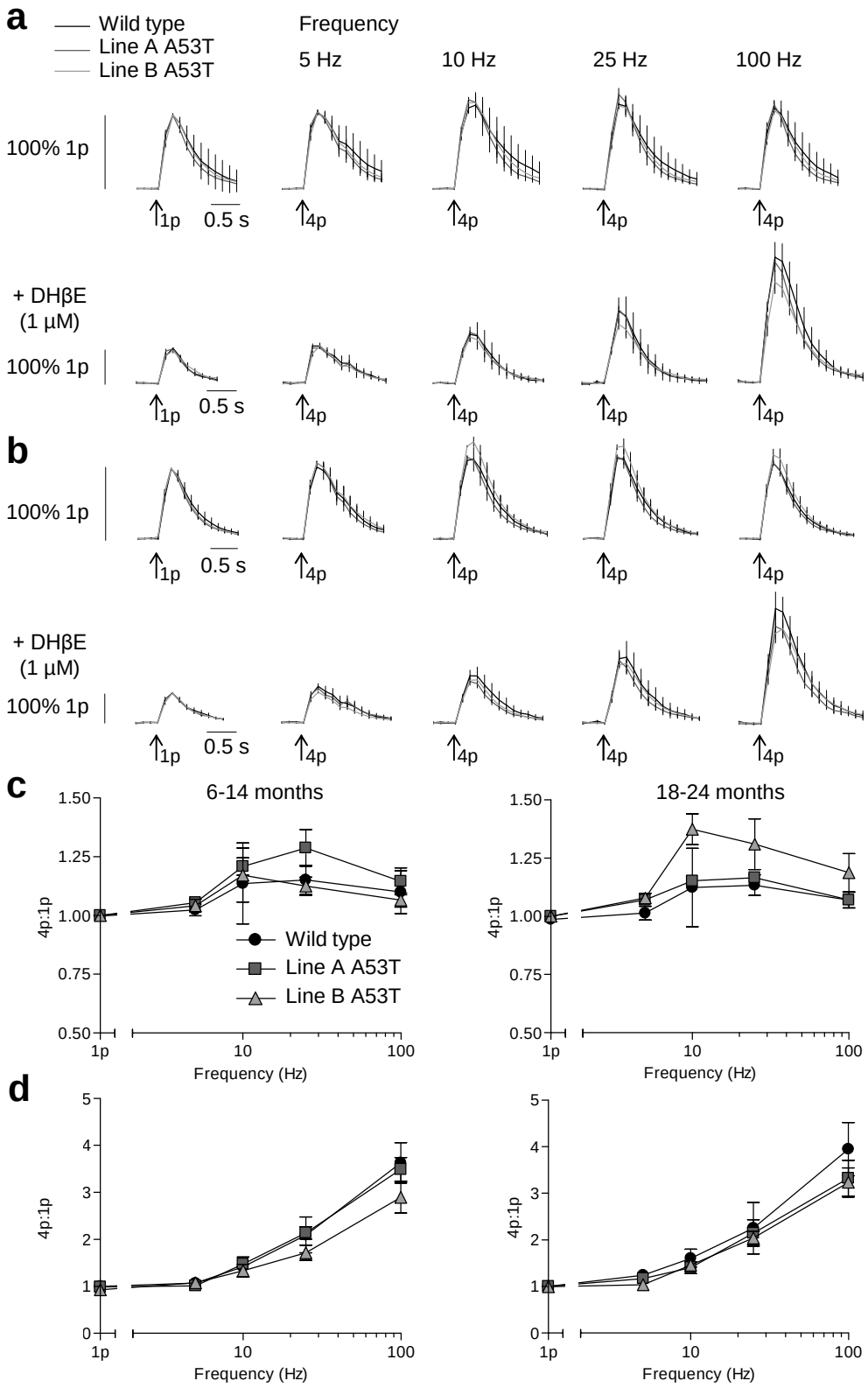

Figure 6.5. A53Tα-syn overexpression does not greatly affect the frequency response of DA release. (a,b) Profiles of mean $[DA]_o$±SEM evoked by single pulse or train stimulations of different frequencies (1p or 4p, 5-100 Hz, arrow), against time, without (upper), and with (lower) nAChR inhibition (DHβE, 1 μM), at 6-14 months **(a)** and 18-24 months **(b)**. **(c,d)** Mean 4p:1p±SEM without (c) and with (d) nAChR inhibition (DHβE, 1 μM). In drug-free conditions (c), there was no interaction between genotype and frequency but there was a main effect of genotype at 18-24 months (Two-way ANOVA, 6-14 months: main effect of genotype $p > 0.05$ $F_{2,40}$=1.39, main effect of frequency $p < 0.01$ $F_{4,40}$=4.94, interaction $p > 0.05$ $F_{8,40}$=0.32; 18-24 months: main effect of genotype $p < 0.01$ $F_{2,40}$=7.66, main effect of frequency $p < 0.001$ $F_{4,40}$=9.63, interaction $p > 0.05$ $F_{8,40}$=1.06; $n = 3$-5). With DHβE, there was no significant effect of genotype on 4p:1p at either age Two-way ANOVA, 6-14 months: main effect of genotype $p > 0.05$ $F_{2,40}$=2.55, main effect of frequency $p < 0.01$ $F_{4,40}$=75.70, interaction $p > 0.05$ $F_{8,40}$=0.77; 18-24 months: main effect of genotype $p > 0.05$ $F_{2,40}$=1.29, main effect of frequency $p < 0.001$ $F_{2,40}$=44.43, interaction $p > 0.05$ $F_{8,40}$=0.33; $n = 3$-5).

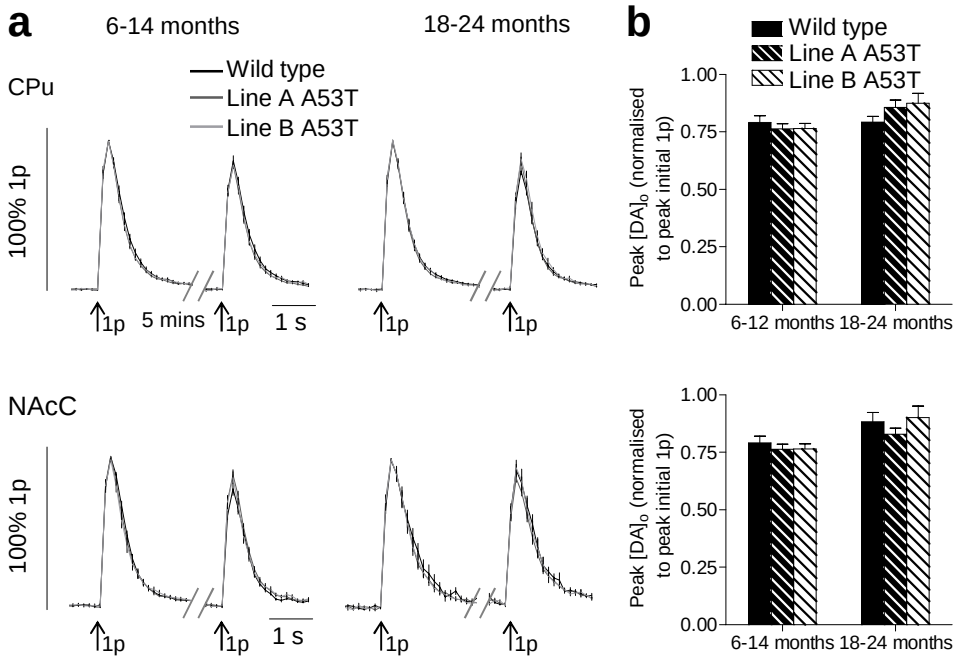

Figure 6.6 A53Tα-syn overexpression does not affect subsequent DA release minutes after initial stimulation. (a) Profiles of mean $[DA]_o$±SEM against time, evoked by two 1p stimulations, separated by 5 minutes. There was no significant difference between genotypes in normalised $[DA]_o$ evoked by a second 1p, at either age, in either region One-way ANOVA, CPu: 6-14 months *p>0.05*, $F_{2,57}$=0.03; 18-24 months *p>0.05*, $F_{2,57}$=0.03; *n=15-40*, NAcC: 6-14 months *p>0.05*, $F_{2,57}$=0.05; 18-24 months *p>0.05*, $F_{2,57}$=0.04; *n=6-14*). **(b)** Mean peak $[DA]_o$±SEM evoked by second 1p, normalised to peak $[DA]_o$ evoked by initial 1p.

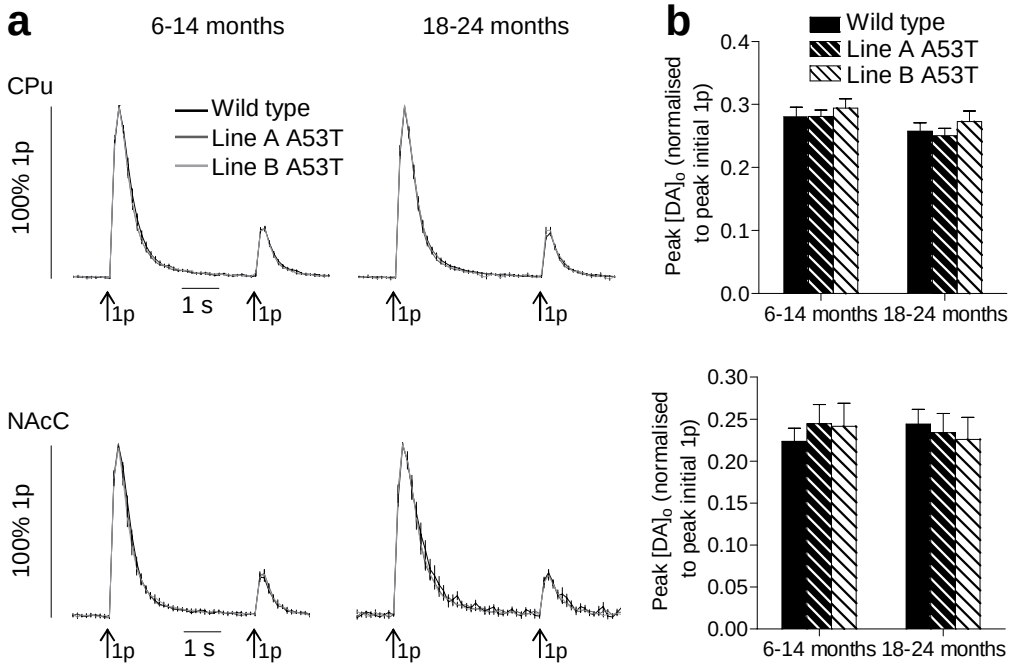

Figure 6.7. A53Tα-syn overexpression does not affect the short-term depression of release. (a) Profiles of mean $[DA]_o$±SEM versus time, evoked by two 1p stimulations, separated by 4 s. There was no significant difference in peak normalised $[DA]_o$ evoked by a second 1p between genotypes, at either age, in either region (One-way ANOVA, CPu: 6-14 months *p>0.05*, $F_{2,93}$=0.01; 18-24 months *p>0.05*, $F_{2,182}$=0.01; *n=15-40*, NAcC: 6-14 months *p>0.05*, $F_{2,175}$=0.00; 18-24 months *p>0.05*, $F_{2,182}$=0.08; *n=6-14*). **(b)** Mean peak $[DA]_o$±SEM evoked by second 1p, normalised to peak $[DA]_o$ evoked by initial 1p.

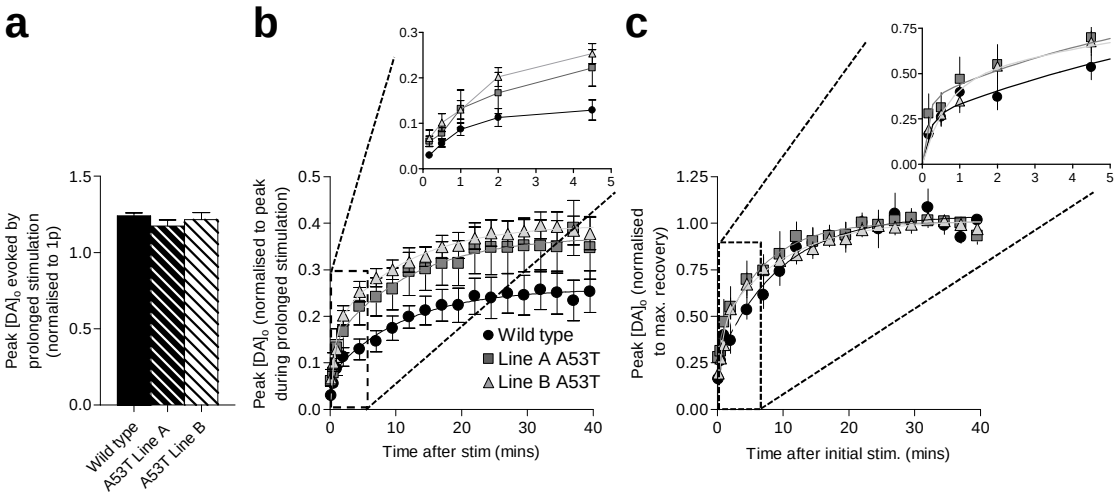

Figure 6.8. A53Tα-syn overexpression enhances recovery of DA release following prolonged stimulation. **(a)** Mean peak $[DA]_o$±SEM evoked during a prolonged stimulation train (60 s, 10 Hz), normalised to mean peak $[DA]_o$ evoked by 1p. There was no significant difference in peak $[DA]_o$ evoked by prolonged stimulation between genotypes (One-way ANOVA, $p > 0.05$ $F_{2,24}$=0.80; $n = 8$-10). **(b,c)** Mean peak $[DA]_o$±SEM evoked by 1p, against time after the end of prolonged stimulation. Values were normalised to **(b)** peak $[DA]_o$ evoked by prolonged stimulation or **(c)** maximum recovery of peak 1p $[DA]_0$ after prolonged stimulation. Curve fits are two phase exponential associations, $R^2>0.80$. Both sets of curves are significantly different between genotypes ((b): $p < 0.001$ $F_{8,48}$=180.5, (c): $p < 0.001$ $F_{8,48}$=4.31; $n = 8$-10). Inset is the expanded initial 5 minutes following the end of prolonged stimulation.

7. Discussion

Striatal DA plays important roles in many behaviours, and dysfunction of striatal DA signalling is implicated in the pathogenesis of a number of disorders. Much is known about the behavioural correlates of different DA neuron firing patterns, and these patterns are often assumed to correlate with striatal DA function. However, striatal DA release, and hence its downstream effects, are highly dynamic and depend on many local factors in addition to DA neuron firing pattern. Hence it is necessary to understand the local striatal control of DA release and short-term plasticity of release to fully understand the behavioural relevance of DA neuron firing patterns. This thesis has identified a number of mechanisms which interact to control striatal DA release and the short-term plasticity of release, and which may have implications for the actions of drugs of abuse, and PD pathology. This chapter will consolidate these findings, discuss what they tell us about the control of striatal DA release and consider their behavioural and therapeutic implications.

7.1 P_r plays a relatively minor role in the control of the short-term plasticity of striatal DA release

In chapter 3, the basic characteristics of the short-term plasticity of DA release were explored, particularly focussing on whether P_r is an important determinant of the short-term plasticity of striatal DA release. In many central synapses, P_r plays a significant role in determining the short-term plasticity of release, since a greater P_r means that an initial action potential releases more vesicles, leaving fewer docked and immediately releasable vesicles available for subsequent release (Thomson *et al.* 1993; Dobrunz and Stevens 1997; Thomson 1997; Thomson 2000). However, it was unknown if similar mechanisms control the short-term plasticity of striatal DA release. The data presented in chapter 3 suggest that P_r plays only a minor role in the control of the short-term plasticity of striatal DA release. When nAChRs are active, P_r had almost no effect on the short-term plasticity of DA release. When nAChRs are not active, there was a classic

inverse relationship between P2/P1 and P_r, suggesting that this release-dependent mechanism of short-term plasticity does indeed exist and contribute to the control of transmitter release in DA neurons. However, this relationship was only seen at very short IPIs, which are only occasionally reported in DA neurons *in vivo* (Grace and Bunney 1984; Hyland *et al.* 2002). Additionally, manipulating $[K^+]_o$ showed that P_r and the short-term plasticity of release are dissociable, even at short IPIs when nAChRs are inhibited. Overall then, P_r is unlikely to play a large role in the control of DA release plasticity *in vivo*.

Instead, DA release was found to exhibit strong, release-independent depression at IPIs longer than approximately 40 ms. DA release has previously been reported to recover from this depression over a time course of around 30-60 seconds (Abeliovich *et al.* 2000). The mechanisms underlying this form of depression are unclear. Although D_2 autoreceptor feedback control of DA release may contribute, this cannot account entirely for the depression, since D_2Rs only strongly control subsequent DA release at IPIs longer than around 250 ms (Benoit-Marand *et al.* 2001; Phillips *et al.* 2002; Schmitz *et al.* 2002), and strong depression is still observed in the presence of D_2R antagonists (see **Fig. 4.10, 4.11**, Mayer *et al.* 1988; Kennedy *et al.* 1992; Abeliovich *et al.* 2000; Cragg 2003). It is possible that activity-dependent changes in ion conductances, particularly K^+ conductances, may contribute to this depression (Dodson and Forsythe 2004), although such changes in conductance are usually proposed to modulate release over millisecond timescales (Thomson 2000; Dodson and Forsythe 2004; Bucher and Goaillard 2011), whereas DA release recovers from depression over approximately 30 s (Abeliovich *et al.* 2000). Another possible mechanism may involve the selective 'switching off' of synapses, such that individual release sites function in an all-or-none manner (Royer *et al.* 2000). Additionally, results described in chapter 4 suggest that the DAT may play a role in this release-independent clamping of subsequent release. This is discussed further below.

7.1.1 Implications

P_r varies significantly between DA neuron terminals, and particularly between striatal regions (Cragg 2003; Daniel *et al.* 2009). P_r has also been suggested to be modulated by a number of factors which may be relevant to disorders such as PD and drug addiction, including α-syn expression (Nemani *et al.* 2010), Ca^{2+} channel and Ca^{2+}-binding protein expression (Atwood and Karunanithi 2002) and drugs of abuse such as cocaine (Venton *et al.* 2006). The results described here suggest that, although such factors may influence absolute levels of DA release, they will not necessarily strongly influence the short-term plasticity of release, and may therefore have little impact on the contrast between striatal DA signals evoked by high frequency burst firing vs. low frequency firing. As described in chapter 1.1.3.3, the switch from low frequency firing to high frequency burst firing in DA neurons is thought to encode behaviourally important, reward-related information (Schultz 2007). Therefore, the release-independent nature of the short-term plasticity of DA release may function to maintain transmission of this information to postsynaptic neurons, in spite of presynaptic perturbations which may change levels of DA release.

7.2 nAChRs strongly control the short-term plasticity of striatal DA release, independently of their effect on P_r

ACh released from ChIs, can act at nAChRs on DA neuron axons or presynaptic terminals to directly drive DA release, even in the absence of DA neuron activity (Threlfell *et al.* 2012) (see **Fig 1.4**). Additionally, nAChRs are known to play a highly significant role in controlling the short-term plasticity of DA release (Rice and Cragg 2004; Zhang and Sulzer 2004). When nAChRs are active, DA release shows strong paired-pulse depression. When nAChRs are inhibited, depression is relieved and DA release shows facilitation at very short IPIs, which decays over 25-100 ms (see **Fig. 1.5**). It has previously been assumed that these two effects of nAChRs, driving DA release and modulating the short-term plasticity of release, are interconnected: nAChRs act to drive or

increase DA release, leaving fewer vesicles available for subsequent release, and hence causing short-term depression (Zhang and Sulzer 2004; Threlfell *et al.* 2010). However, the results presented in chapter 3 suggest that these two effects of nAChRs are independent. nAChR inhibition altered absolute levels of DA release comparably to other manipulations of P_r, or to variations in DA release between recording sites. However, in contrast to other manipulations, nAChR inhibition greatly modulated the short-term plasticity of DA release, switching it from strong depression across all IPIs to facilitation at short IPIs which decayed gradually over several hundred milliseconds (Rice and Cragg 2004; Zhang and Sulzer 2004).

In addition, nAChRs were found to interact with P_r in the control of DA release plasticity. As described in 7.1, when nAChRs were active, other manipulations of P_r had no effect on the short-term plasticity of release. However, when nAChRs were inhibited, there was an inverse relationship between P2/P1 and P_r, albeit only visible at short IPIs.

Together, these results suggest that ACh does not simply act at nAChRs to increase P_r and drive DA release, and hence modify the short-term release-dependent plasticity of release, but fundamentally changes how DA terminals filter firing patterns into DA release. nAChRs appear to clamp release, strongly reducing subsequent release following an initial action potential or following an initial ACh-evoked release event, and making subsequent release invariant with P_r. The mechanism for this clamping is uncertain. Feasible mechanisms include protein-protein interactions of nAChRs with Ca^{2+}-sensitive presynaptic proteins, or changes in action potential conduction.

7.2.1 Implications

This clamping of release has important implications for the role of striatal cholinergic signalling and for the actions of the addictive drug nicotine. ChIs tend to show a burst-pause-burst pattern of activity in response to behaviourally salient events, coincident with the switch from tonic to burst firing in DA neurons (Morris *et al.* 2004). The results presented in chapter 3 support the

hypothesis that the pause in ChI firing, thought to reduce striatal ACh levels and hence nAChR activation, will permit the switch in DA neuron firing pattern to be effectively translated into a large change in striatal DA release (Cragg 2006). In addition, since DA neuron firing rate is often greatest, and hence IPI is smallest, during ChI pauses (Morris *et al.* 2004), changes in P_r may subtly modify the short-term plasticity of DA release during this period. Therefore, during the ChI pause, information carried by both the firing rate and P_r of DA neurons will be effectively transmitted to postsynaptic cells. In contrast, during the flanking periods of ChI excitation, when ACh release is thought to increase and drive DA release, DA release evoked by subsequent action potentials in DA neurons will be strongly clamped. Action potentials in DA neurons will release very little DA on top of that evoked by ACh, and this will be unmodulated by P_r. Therefore, in these conditions, striatal DA will actually convey information carried in ChIs rather than that carried in DA neurons. In this way, the bursts in ChIs, which flank the characteristic pause in activity, may be behaviourally significant. Indeed, the rebound excitation has been proposed to be more plastic and hence carry more reward-related information than the pause itself (Joshua *et al.* 2008; Apicella *et al.* 2011). This information may be transmitted to postsynaptic cells by changes in DA release as well as changes in ACh release.

These findings also have implications for the actions of nicotine. At levels found in smokers, nicotine is thought to desensitise nAChRs on DA terminals, and hence function like a nAChR antagonist (Zhou *et al.* 2001; Rice and Cragg 2004). The results presented in chapter 3 suggest that nicotine will therefore fundamentally change how DA neuron terminals filter firing patterns into striatal DA release, in addition to, and independently of, its effects on absolute levels of DA release. By desensitising nAChRs, nicotine will permit the translation of changes in DA neuron firing and DA P_r into changes in DA release. Hence it will increase the transmission of information carried by DA neurons to postsynaptic cells, even when ChIs are active. DA neurons carry primarily reward-related information, whereas ChIs are thought to carry more salience-related information (Ravel *et al.* 1999; Morris *et al.* 2004; Apicella *et al.* 2011). Therefore, disrupting the

interaction between these two systems will alter the information received by postsynaptic striatal cells, and hence may disrupt reward processing and reinforcement learning. This may contribute to the disrupted reward processing caused by this addictive drug (Mendez *et al.* ; Kolokotroni *et al.* 2011; Mitchell *et al.* 2011).

7.3 The DAT directly controls the short-term plasticity of striatal DA release

In chapter 4 a role for another presynaptic factor, the DAT, in controlling the short-term plasticity of DA release was identified. The DAT removes DA from the extracellular space by secondary active transport, to be repackaged and recycled for re-release. The data presented in chapter 4 suggest that, when nAChRs are not active, the DAT also functions to control the short-term plasticity of DA release. DAT inhibition greatly reduced the range of striatal DA signals evoked by train stimulations of different frequencies, decreasing the contrast between DA release evoked by high vs. low frequency activity. This was due to changes in the short-term plasticity of DA release, in addition to changes in the extracellular summation of DA. DAT inhibition, or other models of decreased DAT activity, strongly decreased subsequent release at very short IPIs after initial release, but, in dlCPu, appeared to increase subsequent release at slightly longer IPIs. This suggests that DAT activity promotes facilitation of release at short IPIs, less than 25-40 ms, but, in dlCPu, slightly promotes depression of release at longer IPIs, 25-200 ms.

Potential mechanisms underlying this effect of the DAT were explored. The effect of DAT inhibition on the short-term plasticity of DA release was similar at a 1st and 2nd stimulation epoch, when it is hypothesised that levels of DA transport via the DAT will differ substantially. This suggests that this role of the DAT is not dependent on DA transport itself. D_2R antagonists did not modulate the effects of DAT inhibition on the short-term plasticity of DA release, suggesting that activation of D_2Rs also does not play a role in these actions of the DAT. This is as expected since

D_2Rs are only thought to control DA release over a time course of approximately 250 ms to several seconds after initial release (Benoit-Marand *et al.* 2001; Phillips *et al.* 2002; Schmitz *et al.* 2002). Modulating $[Cl^-]_o$ also did not modulate the effects of the DAT on the short-term plasticity of DA release, suggesting that the ion channel-like Cl^- conductance of the DAT (Ingram *et al.* 2002) is not involved in this function of the DAT.

The two opposing effects of the DAT on the short-term plasticity of DA release at different IPIs, in dlCPu, could be dissociated using a low concentration of cocaine, suggesting that different underlying mechanisms may cause these two effects. This is further supported by the fact that lowering $[Ca^{2+}]_o$ increased the effect of DAT inhibition at longer IPIs, but did not modulate the effect of DAT inhibition at very short IPIs, again suggesting these two effects may occur via different mechanisms. This effect of modulating $[Ca^{2+}]_o$ was not simply due to changes in P_r, since decreasing P_r by decreasing stimulation current did not modulate the effect of DAT inhibition. Therefore, the effect of the DAT on the short-term plasticity of DA release in dlCPu over 25-200 ms IPIs occurs via a Ca^{2+}-dependent mechanism. The effect of DAT inhibition to increase initial DA release via a synapsin-dependent mechanism (Venton *et al.* 2006; Kile *et al.* 2010) has also been reported to be increased with decreased $[Ca^{2+}]_o$ (Kile *et al.* 2010). Therefore, it is possible that this Ca^{2+}- and synapsin-dependent mechanism underlying the DAT control of initial release also underlies the effect of the DAT on the short-term plasticity of release at longer IPIs.

The mechanism underlying the effect of the DAT on the short-term plasticity of DA release at very short IPIs is unclear. It may feasibly be due to the effect of the DAT on levels of initial DA release, as discussed in chapter 3 and 7.1. DAT inhibition is thought to mobilise a synapsin-dependent reserve pool of vesicles, as discussed above (Venton *et al.* 2006), increasing initial release, which may decrease the number of vesicles available for subsequent release and hence decrease P2/P1 at short IPIs. Conversely, it may be predicted that DAT activity reduces or limits initial release, which would leave many readily releasable vesicles available for subsequent

release, permitting facilitation of release at short IPIs. However, results from chapter 3 suggest that increasing P_r has relatively minor effects on the short-term plasticity of DA release. In contrast, the DAT appears to strongly control the short-term plasticity of DA release at very short IPIs, to a greater extent than any manipulation of P_r in chapter 3. Additionally, the effect of the DAT on the short-term plasticity of DA release at short IPIs does not depend on $[Ca^{2+}]_o$, whereas the synapsin-dependent control of initial DA release has been reported to do so, as described above (Kile *et al.* 2010). Therefore, the effect of the DAT on the short-term plasticity of DA release at very short IPIs may occur via a different mechanism, possibly involving other protein-protein interactions of the DAT.

The synapsin isoform proposed to be involved in the effects of DAT inhibition on DA release is synapsin III (Kile *et al.* 2010). However, data presented in chapter 4 showed that the effects of DAT inhibition on both P_r and the short-term plasticity of DA release appear to be independent of synapsin III. Therefore, if the effects of DAT inhibition do depend on the control of a vesicle reserve pool, it may be a synapsin I- or II-dependent pool.

It is further interesting to note that modulating $[Ca^{2+}]_o$ significantly modulated the short-term plasticity of DA release at longer IPIs when the DAT was inhibited (chapter 4), in contrast to when the DAT was active (chapter 3). Therefore, the activity of the DAT may contribute to the release-independent depression of DA release at these longer IPIs, demonstrated in chapter 3 and discussed above. However, it cannot fully account for the release-independent depression, since in chapter 4 it was shown that modulating stimulation current still did not modulate the short-term plasticity of release at longer IPIs, even when the DAT was inhibited.

7.3.1 Implications

The results presented in chapter 4 suggest that the endogenous activity of the DAT modulates the short-term plasticity of DA release. This is an intriguing and previously unconsidered role for an uptake transporter. Many neurotransmitters have specific uptake transporters structurally or

functionally similar to the DAT (Gainetdinov and Caron 2003) and it would therefore be interesting to explore whether the control of release plasticity is a more generalized role for transporters.

The results in chapter 4 suggest that, when nAChRs are not active, the DAT acts to increase the range of the short-term plasticity of DA release, increasing the contrast between DA release evoked by high frequency vs. low frequency activity. Additionally, it has been previously shown that uptake via the DAT more strongly limits the extracellular summation of DA signals evoked by low frequency vs. high frequency firing (Garris and Rebec 2002). Therefore together, the effects of the DAT on extracellular summation and the effects of the DAT on DA release, described here, will function to promote the contrast between striatal DA signals evoked by high frequency bursts vs. low frequency tonic firing, when nAChRs are not active. As discussed previously, switches in DA neuron firing from low frequency tonic firing to high frequency burst firing, are thought to encode behaviourally salient, particularly reward-related information (Schultz *et al.* 1997). The activity of the DAT therefore appears to be another mechanism via which DA terminals permit these behaviourally related switches in firing pattern to be translated into large changes in striatal DA release. Such large changes in DA release will be easily detected by postsynaptic DA target neurons, and are predicted to promote synaptic plasticity in these cells. Therefore, DAT activity may be hypothesised to allow the information carried by DA neuron firing to be efficiently transmitted to postsynaptic striatal cells, which may be important for reward-related behaviour and learning.

These results may have implications for the actions of drugs of abuse, particularly psychostimulants such as cocaine. The results presented in chapter 4 suggest that such drugs will reduce the contrast between reward-related and tonic striatal DA signals, when nAChRs are not active, and hence will impair transmission of reward-related information to postsynaptic cells.

This may contribute the impairment of reward-related decision making seen with these drugs (Grant *et al.* 2000; Simon *et al.* 2007; Porter *et al.* 2011).

7.4 The indirect role of the DAT in controlling the short-term plasticity of DA release occurs with different efficacy in dorsal vs. ventral striatum

The results presented in chapter 4 and discussed above demonstrate a direct role for the DAT in controlling the short-term plasticity of DA release, when nAChRs are not active. However, previous work in our lab has demonstrated that the DAT plays another role in controlling DA release and the short-term plasticity of release, via control of D_2R activation, when nAChRs are active (Clements *et al. In preparation*). At short intervals, up to 2 s, following previous electrical stimulation, DA release evoked by a single stimulus pulse is strongly depressed, but the short-term plasticity of DA release is modulated such that the sensitivity of release to high frequency stimulation is enhanced. This increased frequency sensitivity is dependent on D_2R activation. The DAT controls the extent of this D_2R-mediated effect; DAT inhibitors increase the amplitude and lifetime of extracellular DA signals, increasing and prolonging D_2R activation and hence increasing and prolonging the modulation of the short-term plasticity of DA release (Clements *et. al* in preparation).

The data presented in chapter 5 demonstrate that this effect of DAT inhibition on the short-term plasticity of DA release occurs with greater efficacy in NAcC vs. dlCPu, despite comparable effects on DA uptake and DA release evoked by a single stimulus pulse. Since this effect of DAT inhibition depends on D_2Rs, the relative efficacy of D_2R control of DA release in the two striatal regions was then explored. D_2 autoreceptors on DA terminals are known to play a significant role in controlling DA release (Schmitz *et al.* 2002; Bello *et al.* 2011). However, it has been suggested that D_2 heteroceptors may also contribute to the D_2R control of striatal DA release (Anzalone *et*

al. 2012). In particular, D_2Rs on ChIs are ideally placed to indirectly control DA release. Activation of D_2Rs on ChIs inhibits ChI firing and ACh release (DeBoer and Abercrombie 1996; Yan *et al.* 1997; Pisani *et al.* 2000), and hence is hypothesised to decrease nAChR activation, modulating DA release and the short-term plasticity of release. In chapter 5 an optogenetic approach was used to begin to dissect these components of D_2R control of DA release, and allow them to be compared between striatal regions. D_2 autoreceptor control of DA release was found to occur with similar efficacy in both striatal regions. In contrast, D_2R control of ACh-evoked DA release occurred with greater efficacy in NAcC vs. dlCPu, suggesting this may underlie the regional difference in the effects of cocaine on the short-term plasticity of DA release. D_2R control of ACh-evoked DA release most likely involves a compound effect of both D_2Rs on DA terminals and D_2Rs on ChIs. Since D_2 autoreceptor control does not differ greatly between striatal regions, these data suggest that D_2Rs on ChIs differ in efficacy of control of DA release between striatal regions. The mechanism underlying this may involve different efficacy of D_2R coupling to downstream pathways and hence different D_2R control of ACh release, or alternatively may reflect regional differences in the efficacy of ACh signalling, for example differences in ChI packing densities, AChE expression or nAChR efficacy.

7.4.1 Implications

7.4.1.1 Differential efficacy of D_2R control of DA release may contribute to the preferential increase of DA in ventral vs. dorsal striatum with cocaine

It is well documented that the DAT inhibitor cocaine preferentially increases extracellular DA in the ventral vs. dorsal striatum (Carboni *et al.* 1989; Cass *et al.* 1992; Kuczenski and Segal 1992; Wu *et al.* 2001). This regional difference has been suggested to be due to differences in uptake between the two regions (Cass *et al.* 1992), a compound effect of differential release and uptake (Wu *et al.* 2001) or a different effect of cocaine on the incidence of release events (Aragona *et al.* 2008). The results presented in chapter 5 suggest that, when nAChRs are active, cocaine preferentially increases the frequency sensitivity of DA release, increasing the contrast between

DA signals evoked by high vs. low frequency activity, in the NAcC vs. dlCPu. This is an additional mechanism which may contribute to the greater increase of extracellular DA in ventral vs. dorsal striatum with cocaine. Also, as discussed previously, the switch from low frequency tonic to high frequency burst activity in DA neurons is thought to encode reward-related information. By increasing the frequency sensitivity of release, DAT inhibitors will promote the translation of these different firing patterns into large changes in DA release when nAChRs are active, preferentially in NAcC vs. dlCPu. Therefore, DAT inhibitors will promote the transmission of reward-related information to postsynaptic cells preferentially in NAcC vs. dlCPu. This may be significant in the strongly reinforcing effects of the drug, since reinforcement learning, which is dependent on reward-related information, particularly involves DA signalling in the ventral, rather than dorsal, striatum (Everitt and Wolf 2002).

7.4.1.2 DAT inhibitors disrupt reward-related signalling in the striatum

Together, the results presented in chapters 4 and 5 demonstrate that the DAT plays a significant role in controlling the short-term plasticity of DA release, with opposing effects when nAChRs are active and inactive. Chapter 3 demonstrated that nAChRs are a key determinant of DA release plasticity, and determine whether reward-related information carried by DA neurons is effectively translated into changes in striatal DA signals and hence transmitted to postsynaptic cells. When nAChRs are not active, hypothesised to be during pauses in ChI firing, the switch from low to high frequency DA neuron firing will be translated into a large change in extracellular DA. When nAChRs are active, hypothesised to be during rebound excitation in ChIs, DA release will be clamped and information carried by DA neuron firing will not be translated into changes in striatal DA release. The DAT appears to play a significant role in maintaining this different filtering of firing patterns into striatal DA release. Chapter 4 demonstrated that when nAChRs are not active, the DAT promotes the contrast between DA signals evoked by high vs. low-frequency activity. In contrast, when nAChRs are active, the DAT limits the extent of D_2R activation, and hence limits the contrast between DA signals evoked by high vs. low-frequency activity (Clements

et al. In preparation) chapter 5). This has important implications for the actions of DAT inhibitors, suggesting that such drugs will disrupt the transmission of reward-related information carried by DA neuron firing, both when nAChRs are active and inactive. This may contribute to the behavioural effects of such drugs. For example, it may contribute to the impairment in reward-related decision making caused by cocaine (Grant *et al.* 2000; Simon *et al.* 2007; Porter *et al.* 2011). In contrast, in patients with ADHD, who are hypothesised to have baseline differences in dopaminergic signalling pathways, including in DAT expression or function (Cook *et al.* 1995; Comings *et al.* 1996; Dougherty *et al.* 1999; Sakrikar *et al.* 2012), DAT inhibitors may normalise the striatal processing of salience- and reward-related information.

7.5 Mutant α-syn has only subtle effects on striatal DA release

As discussed in chapter 1.2, a large and complex array of proteins is involved in the control of transmitter release and the short-term plasticity of release. Interactions with these presynaptic proteins may contribute to the effects of nAChRs, the DAT, and D_2Rs on the short-term plasticity of DA release. α-syn is one such protein. α-syn is a presynaptic protein that has been implicated in the control of vesicle trafficking , release, and the short-term plasticity of release (Abeliovich *et al.* 2000; Murphy *et al.* 2000; Nemani *et al.* 2010; Anwar *et al.* 2011), and has been shown to interact functionally with the DAT and D_2Rs (Sidhu *et al.* 2004; Jae Kim *et al.* 2006; Fountaine *et al.* 2008; Graham and Sidhu 2010). Additionally, the effects of mutant α-syn on DA release are of interest due to the strong implication of α-syn in the pathogenesis of PD. A53Tα-syn causes dominantly inherited PD in humans (Polymeropoulos *et al.* 1997) and animals overexpressing A53Tα-syn show abnormal cellular accumulation of α-syn, deficits in striatal long-term synaptic plasticity, and behavioural deficits consistent with a hypodopaminergic phenotype, prior to overt nigrostriatal degeneration (Gispert *et al.* 2003; Kurz *et al.* 2010). These results suggested that in this animal model of early PD there may be changes in striatal DA release, even before

nigrostriatal degeneration. Therefore, in chapter 6, FCV was used to explore directly whether the overexpression of A53Tα-syn modulates striatal DA release and the short-term plasticity of release.

A53Tα-syn overexpression had little effect on most parameters of striatal DA release. Gross levels of DA release and uptake were similar in WT and A53Tα-syn overexpressors, in both dorsal and ventral striatum. However, since A53Tα-syn overexpressors have increased striatal DA content (Kurz *et al.* 2010), this may actually reflect a slight decrease in the releasability of striatal DA. The frequency sensitivity of DA signals, both when nAChRs were active and inhibited, was also similar in A53Tα-syn overexpressors vs. WTs. Additionally, DA release several seconds or minutes after an initial single stimulation was unchanged by A53Tα-syn overexpression. Together these results suggest that A53Tα-syn does not strongly control the short-term plasticity of DA release. However, following prolonged stimulation, DA release recovered more quickly and to a greater extent in A53Tα-syn overexpressors vs. WTs. This may be caused by changes in vesicle trafficking and clustering, or altered expression of presynaptic chaperone proteins, which have previously been reported to result from the overexpression of mutant α-syn (Abeliovich *et al.* 2000; Murphy *et al.* 2000; Cabin *et al.* 2002; Kurz *et al.* 2010). However, in cultured neurons, mutant α-syn has previously been reported to impair rather than enhance vesicle clustering and release (Nemani *et al.* 2010). The increase in release following prolonged stimulation in A53Tα-syn overexpressors reported in chapter 6 may therefore reflect compensatory mechanisms found *in vivo*, for example to compensate for the slight decrease in releasability.

7.5.1 Implications

Although A53Tα-syn overexpression has only subtle effects on striatal DA release, it is possible that over the lifetime of the animal, with continuous DA neuron activity and DA release, these subtle changes in striatal DA handling contribute to the late onset postsynaptic and behavioural deficits observed in these animals (Gispert *et al.* 2003; Kurz *et al.* 2010; Tozzi *et al.* 2011). Much

recent interest has focussed on a presynaptic locus for early pathological changes in DA neurons in PD prior to overt neurodegeneration (Cheng *et al.* 2010; Lundblad *et al.* 2012). The results presented in chapter 6 support the hypothesis that subtle presynaptic changes may occur in nigrostriatal DA neurons early in PD, before DA neurons in the midbrain begin to die.

The subtle effects of A53Tα-syn on striatal DA release are consistent with previous reports that overexpression of another mutant α-syn in mice, A30Pα-syn, causes only subtle changes in striatal DA release *in vivo* (Yavich *et al.* 2005). In contrast, mutant α-syn has been reported to have a large impact on transmitter release in cultured neurons or non-neuronal cells (Park *et al.* 2002; Larsen *et al.* 2006; Nemani *et al.* 2010). This relatively subtle effect *in vivo* compared to *in vitro* may reflect many compensatory mechanisms present *in vivo* which act to normalise DA release in the face of presynaptic perturbation. Such compensatory mechanisms may contribute to the relatively late onset of the disease in both animal models and in humans. It is feasible that the increased striatal DA content in A53Tα-syn overexpressors (Kurz *et al.* 2010), and the increased recovery of release following prolonged stimulation reported in chapter 6, may also reflect compensatory mechanisms to maintain DA release despite a decreased releasability, consistent with a role for α-syn in negatively regulating transmitter release (Senior *et al.* 2008; Anwar *et al.* 2011). This would support the proposal, suggested by the previous chapters of this thesis, that DA terminals express a number of mechanisms which maintain DA release and the short-term plasticity of release in the face of presynaptic perturbations.

α-syn has been shown to functionally interact with the DAT and D_2Rs (Sidhu *et al.* 2004; Jae Kim *et al.* 2006; Fountaine *et al.* 2008; Graham and Sidhu 2010). However, mutant α-syn has only subtle effects on DA release and the short-term plasticity of release compared to the large effect of the DAT and D_2Rs discussed in chapters 4 and 5. Therefore, interactions with α-syn are unlikely to contribute to the effects of the DAT and D_2Rs on DA release and the short-term plasticity of release. It would be interesting to explore the roles of other presynaptic proteins reported to

interact with the DAT or D_2Rs, such as syntaxin-1 or SNAP-25 (Lee *et al.* 2004; Torres 2006), in the control of the short-term plasticity of DA release.

7.6 Presynaptic control of striatal DA release varies subtly with striatal region

As discussed in chapter 1.1, the function of striatal subregions differs due to their differing afferent and efferent connections (Haber 2003). The two major midbrain DA neuron populations, in the SNc and VTA, project preferentially to the dorsal and ventral striatum respectively. These neuronal populations differ in several ways which may affect DA release and release plasticity. For example they differ in Ca^{2+}-binding protein expression, and ion channel expression and function (Gerfen *et al.* 1987; Liss *et al.* 2005; Chan *et al.* 2007; Eulitz *et al.* 2007). Regulation of DA transmission has also previously been shown to differ in the dorsal vs. ventral striatum. For example, the rate of DA uptake (Jones *et al.* 1995), the short-term plasticity of release (Cragg 2003), and the role of synucleins in controlling DA release (Anwar *et al.* 2011) differ with striatal region. Therefore, throughout most of this thesis, the presynaptic control of DA release has been investigated in both dlCPu and NAcC. Presynaptic control of DA release and the short-term plasticity of release has been found to be grossly similar between regions: P_r, nAChRs, the DAT and D_2Rs all control DA release and the short-term plasticity of release in a similar manner in both striatal regions. However, there appear to be subtle regional differences.

Firstly, the time course of the short-term plasticity of DA release may differ slightly between striatal regions. In chapter 3, when nAChRs were inhibited, the short-term facilitation of release appeared to decayed more slowly in NAcC vs. dlCPu, and release-dependent plasticity was apparent at slightly longer IPIs in NAcC vs. dlCPu. This may suggest that release-independent depression may be slightly weaker in NAcC vs. dlCPu. This was supported by data in chapter 5, which showed that D_2 autoreceptor activation increased the burst sensitivity of DA release at 25

Hz stimulation in NAcC but not dlCPu, whereas the burst sensitivity of DA release to 100 Hz burst stimulation was similarly increased in both regions. Therefore, at IPIs representative of DA neuron burst firing *in vivo* (Grace and Bunney 1984; Hyland *et al.* 2002), P_r may play a slightly greater role in controlling the short-term plasticity of DA release in ventral vs. dorsal striatum. However, experiments in chapter 3 directly comparing the effect of $[Ca^{2+}]_o$ on release plasticity in dlCPu vs. NAcC found no difference between the two regions. This may be due to the optogenetic approach that was used in these experiments, for example there may be differences between $DAT^{Cre/+}$ and WT mice, or may suggest that regional differences in the role of P_r are subtle.

Secondly, although not directly compared, the effect, and time course of the effect, of the DAT on the short-term plasticity of DA release, reported in chapter 4, appeared to differ slightly between striatal regions. Overall, the DAT promotes the frequency sensitivity of release in both regions. However, the effect on of the DAT on DA release evoked by a given frequency of activity may vary slightly with region. In dlCPu, DAT inhibition only decreased P2/P1 at the shortest IPI but tended to increase P2/P1 at longer IPIs. In contrast, in NAcC, DAT inhibition decreased P2/P1 at IPIs up to 40 ms but had no effect at longer IPIs. Therefore, the precise effect of the DAT, and hence DAT inhibitors, on DA signalling will depend upon a complex interplay of striatal region and DA neuron firing rate.

Finally, in chapter 5, it was found that the indirect effect of DAT inhibition on DA release occurs with greater efficacy in NAcC vs. dlCPu. This is likely to be at least partly due to a greater efficacy of the D_2R control of ACh-evoked DA release in NAcC vs. dlCPu.

These regional differences in both the direct and indirect effects of DAT inhibition on DA release may contribute to the complex behavioural effects of DAT inhibitor drugs. DAT inhibitors disrupt behaviours dependent both on the ventral striatum, such as reward-related decision making and reinforcement learning (Grant *et al.* 2000; Everitt 2005; Simon *et al.* 2007; Porter *et al.* 2011),

and on the dorsal striatum, such as locomotion and habitual behaviour (Reith *et al.* 1985; Belin and Everitt 2008). However, DAT inhibitors affect these behaviours in different manners and over different time courses; for example, cocaine promotes locomotion but impairs reward-related decision making (Reith *et al.* 1985; Grant *et al.* 2000; Simon *et al.* 2007).

Overall, the mechanisms of control of striatal DA release explored in this thesis appear to be grossly similar between dorsal and ventral striatum but show subtle differences in time course and efficacy. These subtle differences may contribute to the complex behavioural effects of drugs which manipulate striatal DA release.

7.7 Conclusions

7.7.1 The presynaptic control of DA release differs from that reported for other central neurotransmitters

This thesis aimed to investigate the presynaptic and network factors which interact to control striatal DA release. In particular I aimed to explore the control of the short-term plasticity of DA release, since this will determine how behaviourally significant changes in DA neuron firing pattern are filtered into striatal DA release, and hence transmitted to postsynaptic striatal neurons. It can be concluded that the control of DA release and the short-term plasticity of release is very different to that reported for classical central neurotransmitters. Presynaptic factors which greatly control release and release plasticity at other synapses play only minor roles in the control of DA release. For example, mutant α-syn has only subtle effects on striatal DA release whereas in non-dopaminergic cells or in culture mutant α-syn strongly impairs transmitter release (Park *et al.* 2002; Larsen *et al.* 2006; Nemani *et al.* 2010), and P_r plays only a minor role in controlling the short-term plasticity of DA release, whereas it plays a dominant role at other central synapses (Thomson 2000; Zucker and Regehr 2002). In contrast, factors which are not known to strongly modulate release of other central transmitters play a large role in

controlling the short-term plasticity of DA release. These include nAChRs, which not only control DA P_r but dominantly clamp subsequent DA release, the DAT, which directly controls the short-term plasticity of DA release when nAChRs are not active, and D_2Rs, which can control DA release evoked both by DA neuron activity and by ACh, and hence the short-term plasticity of release. These factors will play a large role in determining how DA neuron firing patterns are dynamically filtered into striatal DA release, and hence how reward-related information carried by DA neurons is transmitted to postsynaptic cells. Therefore, the activity of such factors should be taken into account when interpreting the behavioural relevance of DA neuron firing patterns. Additionally, it is important to note that information obtained from the study of other central synapses cannot necessarily be directly applied to the function of striatal DA synapses.

7.7.2 Implications for pathology

The data presented in this thesis may have implications for the actions of drugs of abuse and for understanding the pathological changes in PD.

The results presented here suggest that both nicotine and psychostimulants will severely disrupt information processing in the striatum. As discussed in chapter 1.1 and above, the coincident changes in firing pattern of ChIs and DA neurons carry related but distinct information, with ChIs thought to primarily report salience, with DA neurons carrying more detailed reward-related information (Ravel *et al.* 1999; Morris *et al.* 2004; Schultz 2007; Apicella *et al.* 2011). The balance and interaction between these two striatal systems is thought to be highly important for normal striatal function and behavioural output (Aosaki *et al.* 2010). ChIs, via ACh actions at nAChRs, dominantly control whether changes in DA neuron firing and DA P_r will be translated into changes in striatal DA release, and hence whether information carried by DA neurons will be transmitted to postsynaptic cells. When nAChRs are not active, information carried by DA neurons is readily transmitted into changes in striatal DA release. When nAChRs are active, information carried by DA neurons is not translated into large changes in DA release (Rice and

Cragg 2004) chapter 3). The results presented throughout this thesis suggest that the drugs of abuse nicotine and cocaine will strongly disrupt this interaction, and alter the translation of reward-related information carried by DA neurons into striatal DA release. Nicotine at levels found in smokers is predicted to desensitise nAChRs (Zhou *et al.* 2001; Rice and Cragg 2004). This will allow information carried by DA neuron firing pattern and P_r to be translated into large changes in DA release even when ChIs are active. Cocaine will have opposing effects depending on whether or not nAChRs are active. When nAChRs are active, cocaine will increase D_2R activation on DA terminals and ChIs. This will promote the translation of changes in DA neuron firing into changes in DA release, particularly in the ventral striatum, when the actions of nAChRs should inhibit this (chapter 5). Conversely, when nAChRs are not active and information carried by DA neurons should be readily transmitted to postsynaptic cells, cocaine will inhibit the translation of changes in DA neuron firing into changes striatal DA release (chapter 4). These actions of drugs of abuse are predicted to significantly impair the processing of reward-related information in the striatum, and may contribute to their behavioural effects on decision making and reinforcement learning, which eventually lead to addiction. Opioids have also been proposed to disrupt the translation of DA neuron firing patterns into striatal DA release, via changes in ACh release and nAChR activation (Britt and McGehee 2008). It would be interesting to explore the effects of opioids on DA signalling when nAChRs are inhibited. Furthermore, it would be interesting to explore if the disruption of striatal cholinergic and dopaminergic interactions, and hence the transmission of reward-related information to postsynaptic striatal neurons, is a mechanism common to all addictive drugs.

In contrast to the large impact of these drugs on DA transmission, mutant α-syn, which causes PD in humans (Polymeropoulos *et al.* 1997), was found to have very subtle effects on DA transmission in a mouse model of early PD. This may contribute to the fact that mutant α-syn only causes disease onset in later life, in contrast to drugs of abuse which have immediate and profound behavioural effects. Over many years of continuous DA transmission, the subtle effects

of mutant α-syn on DA release may eventually result in postsynaptic changes and contribute to early PD pathology, prior to overt degeneration.

7.8 Future work

This thesis has identified dominant roles for nAChRs and the DAT in controlling the short-term plasticity of DA release. Heteroceptors and uptake transporters have not been reported to subserve this function at other central synapses. It would be interesting to explore whether these roles are unique to the DA system, or are more general roles for heteroreceptors and transporters in the control of transmitter release. nAChRs are located on the presynaptic terminals of many neuronal types throughout the brain, such as in the hippocampus, where they are known to facilitate and modulate transmitter release (Gray *et al.* 1996; Wonnacott 1997; McKay *et al.* 2007) and are implicated in the pathology of disorders such as Alzheimer's disease (Kása *et al.* 1997). It would therefore be interesting to explore whether nAChRs on other neuronal terminals exert similar profound effects on transmitter release to those reported here. Also, specific uptake transporters exist for the majority of central transmitters, including for glutamate, GABA and the other monoamine transporters. Many of these exhibit ion channel function and can hence modulate membrane potential and neuronal activity (Sonders and Amara 1996; Bagley *et al.* 2005; Wersinger *et al.* 2006). It would therefore be interesting to explore whether these transporters also control transmitter release plasticity. Additionally, unlike the DAT, glutamate and GABA transporters are expressed by postsynaptic and glial cells, and it would be interesting to explore whether they play physiological roles in these cells, in addition to transmitter uptake.

Several of the discussed implications of this thesis depend upon the assumption that behaviourally related changes in ChI firing result in changes in striatal ACh release. However, as has been discussed, transmitter release is highly dynamic. Little is currently known about the

control of ACh release, particularly as it is not currently possible to monitor dynamic subsecond changes in extracellular ACh in the same manner as for DA. Therefore, it may be interesting to explore changes in DA transmission *in vivo*, in combination with ChI recordings, to be able to correlate dynamic changes in DA signalling with changes in ChI firing, and to help explore the behavioural implications of this thesis.

Finally, the mechanisms underlying the profound effects of nAChRs and the DAT on the short-term plasticity of DA release are not fully understood. As discussed in chapter 1.2, large numbers of presynaptic proteins are involved in the control of transmitter release. Data presented in chapter 6 suggest that the presynaptic protein α-syn is not strongly involved in the control of DA release plasticity. It would be interesting to explore however, whether other presynaptic proteins may control the short-term plasticity of DA release and mediate the effects of nAChRs or the DAT via protein-protein interactions. It would also be interesting to explore more directly the effect of nAChRs and the DAT on terminal membrane potential or Ca^{2+} influx, for example in cultured neurons, to help explore further the cellular mechanisms underlying the control of striatal DA release.

Bibliography

Aarsland, D., et al. (2008). Dementia in Parkinson's disease. *Current Opinion in Neurobiology* **21**(6): 676-682.

Abeliovich, A., et al. (2000). Mice lacking [alpha]-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* **25**(1): 239-252.

Adamantidis, A. R., et al. (2011). Optogenetic interrogation of dopaminergic modulation of the multiple phases of reward-seeking behavior. *The Journal of Neuroscience* **31**(30): 10829-10835.

Alberio, T., et al. (2012). Cellular models to investigate biochemical pathways in Parkinson's disease. *FEBS Journal* **279**(7): 1146-1155.

Albin, R., et al. (1989). The functional anatomy of basal ganglia disorders. *Trends in Neurosciences* **12**(10): 366-375.

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

Alexander, G. E., et al. (1991). Basal ganglia-thalamocortical circuits: Parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. *Progress in brain research*. Uylings and Feenstra, Elsevier. **Volume 85:** 119-146.

Alexander, G. E., et al. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Review of Neuroscience* **9**(1): 357-381.

Amejdki-Chab, N., et al. (1992). Effects of several cations on the neuronal uptake of dopamine and the specific binding of [3H]GBR 12783: Attempts to characterize the Na+ dependence of the neuronal transport of dopamine. *Journal of Neurochemistry* **59**(5): 1795-1804.

Anwar, S., et al. (2011). Functional alterations to the nigrostriatal system in mice lacking all three members of the synuclein family. *The Journal of Neuroscience* **31**(20): 7264-7274.

Anzalone, A., et al. (2012). Dual control of dopamine synthesis and release by presynaptic and postsynaptic dopamine D2 receptors. *The Journal of Neuroscience* **32**(26): 9023-9034.

Aosaki, T., et al. (1995). Temporal and spatial characteristics of tonically active neurons of the primate's striatum. *Journal of Neurophysiology* **73**(3): 1234-1252.

Aosaki, T., et al. (1998). Dopamine D1-like receptor activation excites rat striatal large aspiny neurons in vitro. *The Journal of Neuroscience* **18**(14): 5180-5190.

Aosaki, T., et al. (2010). Acetylcholine–dopamine balance hypothesis in the striatum: An update. *Geriatrics & Gerontology International* **10**: S148-S157.

Aosaki, T., et al. (1994). Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning. *The Journal of Neuroscience* **14**(6): 3969-3984.

Apicella, P., et al. (2009). Tonically active neurons in the striatum differentiate between delivery and omission of expected reward in a probabilistic task context. *European Journal of Neuroscience* **30**(3): 515-526.

Apicella, P., et al. (2011). The role of striatal tonically active neurons in reward prediction error signaling during instrumental task performance. *The Journal of. Neuroscience.* **31**(4): 1507-1515.

Aragona, B. J., et al. (2008). Preferential enhancement of dopamine transmission within the nucleus accumbens shell by cocaine is attributable to a direct increase in phasic dopamine release events. *The Journal of Neuroscience* **28**(35): 8821-8831.

Arbuthnott, G. W. and Wickens, J. (2007). Space, time and dopamine. *Trends in Neurosciences* **30**(2): 62.

Arenkiel, B. R., et al. (2007). *In vivo* light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. *Neuron* **54**(2): 205-218.

Atluri, P. P. and Regehr, W.J. (1996). Determinants of the time course of facilitation at the granule cell to purkinje cell synapse. *The Journal of Neuroscience* **16**(18): 5661-5671.

Atwood, H. L. and Karunanithi, S. (2002). Diversification of synaptic strength: Presynaptic elements. *Nature Reviews Neuroscience* **3**(7): 497-516.

Augustin, I., et al. (1999). Differential expression of two novel munc13 proteins in rat brain. *Journal of Biochemistry.* **337**(3): 363-371.

Avshalumov, M. V., et al. (2003). Glutamate-dependent inhibition of dopamine release in striatum is mediated by a new diffusible messenger, H2O2. *The Journal of Neuroscience* **23**(7): 2744-2750.

Bäckman, C. M., et al. (2006). Characterization of a mouse strain expressing Cre recombinase from the 3' untranslated region of the dopamine transporter locus. *Genesis* **44**(8): 383-390.

Bagley, E. E., et al. (2005). GABA transporter currents activated by protein kinase a excite midbrain neurons during opioid withdrawal. *Neuron* **45**(3): 433-445.

Balleine, B. W., et al. (2007). The role of the dorsal striatum in reward and decision-making. *The Journal of Neuroscience* **27**(31): 8161-8165.

Balnave, R. J. and Gage, P. W. (1974). On facilitation of transmitter release at the toad neuromuscular junction. *The Journal of Physiology* **239**(3): 657-675.

Bamford, N. S., et al. (2004). Dopamine modulates release from corticostriatal terminals. *The Journal of Neuroscience* **24**(43): 9541-9552.

Bandopadhyay, R. and de Belleroche, J. (2010). Pathogenesis of Parkinson's disease: Emerging role of molecular chaperones. *Trends in Molecular Medicine* **16**(1): 27-36.

Barbeau, A. (1962). The pathogenesis of Parkinson's disease: A new hypothesis. *Canadian Medical Association Journal* **87**: 802-807.

Barnes, T. D., et al. (2005). Activity of striatal neurons reflects dynamic encoding and recoding of procedural memories. *Nature* **437**(7062): 1158-1161.

Barral, J., et al. (1999). Muscarinic presynaptic inhibition of neostriatal glutamatergic afferents is mediated by Q-type Ca2+ channels. *Brain Research Bulletin* **49**(4): 285-289.

Bass, C. E., et al. (2010). Optogenetic control of striatal dopamine release in rats. *Journal of Neurochemistry* **114**(5): 1344-1352.

Belin, D. and Everitt, B. J. (2008). Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. *Neuron* **57**(3): 432-441.

Bello, E. P., et al. (2011). Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. *Nature Neuroscience* **14**(8): 1033-1038.

Bennett, B. D., et al. (2000). Intrinsic membrane properties underlying spontaneous tonic firing in neostriatal cholinergic interneurons. *The Journal of Neuroscience* **20**(22): 8493-8503.

Bennett, B. D. and Wilson, C. J. (1999). Spontaneous activity of neostriatal cholinergic interneurons in vitro. *The Journal of Neuroscience* **19**(13): 5586-5596.

Bennett, M. R. and Florin, T. (1974). A statistical analysis of the release of acetylcholine at newly formed synapses in striated muscle. *The Journal of Physiology* **238**(1): 93-107.

Benoit-Marand, M., et al. (2001). Inhibition of dopamine release via presynaptic D2 receptors: Time course and functional characteristics *in vivo. The Journal of Neuroscience* **21**(23): 9134-9141.

Bergstrom, B. P. and Garris, P. A. (2003). 'Passive stabilization' of striatal extracellular dopamine across the lesion spectrum encompassing the presymptomatic phase of parkinson's disease: A voltammetric study in the 6-OHDA-lesioned rat. *Journal of Neurochemistry* **87**(5): 1224-1236.

Bergstrom, B. P., et al. (2011). Functional reorganization of the presynaptic dopaminergic terminal in parkinsonism. *Neuroscience* **193**(0): 310-322.

Bernard, V. and Bolam, J. P. (1998). Subcellular and subsynaptic distribution of the NR1 subunit of the nmda receptor GluR2/3 subunit of the AMPA receptor. *European Journal of Neuroscience* **10**(12): 3721-3736.

Bernard, V., et al. (1997). Cellular, subcellular, and subsynaptic distribution of AMPA-type glutamate receptor subunits in the neostriatum of the rat. *The Journal of Neuroscience* **17**(2): 819-833.

Bertran-Gonzalez, J., et al. (2010). What is the degree of segregation between striatonigral and striatopallidal projections? *Frontiers in Neuroanatomy* **4**.136

Betz, W. J. (1970). Depression of transmitter release at the neuromuscular junction of the frog. *The Journal of Physiology* **206**(3): 629-644.

Beuming, T., et al. (2008). The binding sites for cocaine and dopamine in the dopamine transporter overlap. *Nature Neuroscience* **11**(7): 780-789.

Bevan, M. D., et al. (1998). Selective innervation of neostriatal interneurons by a subclass of neuron in the globus pallidus of the rat. *The Journal of Neuroscience* **18**(22): 9438-9452.

Bevan, M. D., et al. (1997). Synaptic integration of functionally diverse pallidal information in the entopeduncular nucleus and subthalamic nucleus in the rat. *The Journal of Neuroscience* **17**(1): 308-324.

Bevan, M. D., et al. (1995). The glutamate-enriched cortical and thalamic input to neurons in the subthalamic nucleus of the rat: Convergence with GABA-positive terminals. *The Journal of Comparative Neurology* **361**(3): 491-511.

Bevan, M. D., et al. (2002). Move to the rhythm: Oscillations in the subthalamic nucleus–external globus pallidus network. *Trends in Neurosciences* **25**(10): 525-531.

Bezard, E., et al. (2000). Adaptive changes in the nigrostriatal pathway in response to increased 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurodegeneration in the mouse. *European Journal of Neuroscience* **12**(8): 2892-2900.

Bi, A., et al. (2006). Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. *Neuron* **50**(1): 23-33.

Binda, A. V., et al. (2005). Regulation of dense core vesicle release from PC12 cells by interaction between the D2 dopamine receptor and calcium-dependent activator protein for secretion (CAPS). *Biochemical Pharmacology* **69**(10): 1451-1461.

Blanchard, V., et al. (1994). Differential expression of tyrosine hydroxylase and membrane dopamine transporter genes in subpopulations of dopaminergic neurons of the rat mesencephalon. *Brain Research. Molecular Brain Research* **22**(1-4): 29-38.

Bogen, I. L., et al. (2006). Absence of synapsin I and II is accompanied by decreases in vesicular transport of specific neurotransmitters. *Journal of Neurochemistry* **96**(5): 1458-1466.

Boja, J. and Kuhar, M. J. (1989). [3H]cocaine binding and inhibition of [3H]dopamine uptake is similar in both the rat striatum and nucleus accumbens. *European Journal of Pharmacology* **173**(2-3): 215-217.

Bolam, J. P., et al. (2000). Synaptic organisation of the basal ganglia. *Journal of Anatomy* **196**(4): 527-542.

Bolam, J. P. and Smith, Y. (1992). The striatum and the globus pallidus send convergent synaptic inputs onto single cells in the entopeduncular nucleus of the rat: A double anterograde labelling study combined with postembedding immunocytochemistry for GABA. *The Journal of Comparative Neurology* **321**(3): 456-476.

Bolam, J. P., et al. (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.

Borgland, S. L., et al. (2004). Acute and chronic cocaine-induced potentiation of synaptic strength in the ventral tegmental area: Electrophysiological and behavioral correlates in individual rats. *The Journal of Neuroscience* **24**(34): 7482-7490.

Borst, J. G. G. and Sakmann, B (1999). Depletion of calcium in the synaptic cleft of a calyx-type synapse in the rat brainstem. *The Journal of Physiology* **521**(1): 123-133.

Boudreau, A. C. and Wolf, M. E. (2005). Behavioral sensitization to cocaine is associated with increased ampa receptor surface expression in the nucleus accumbens. *The Journal of Neuroscience* **25**(40): 9144-9151.

Bouthenet, M.-L., et al. (1991). Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: Comparison with dopamine D2 receptor mRNA. *Brain Research* **564**(2): 203-219.

Boyden, E. S., et al. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. *Nature Neuroscience* **8**(9): 1263-1268.

Boyson, S., et al. (1986). Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in rat brain. *The Journal of Neuroscience* **6**(11): 3177-3188.

Bracci, E., et al. (2002). Dopamine excites fast-spiking interneurons in the striatum. *Journal of Neurophysiology* **87**(4): 2190-2194.

Brischoux, F., et al. (2009). Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. *Proceedings of the National Academy of Sciences* **106**(12): 4894-4899.

Britt, J. P. and McGehee, D. S. (2008). Presynaptic opioid and nicotinic receptor modulation of dopamine overflow in the nucleus accumbens. *The Journal of Neuroscience* **28**(7): 1672-1681.

Brody, D. L. and Yue, D. T. (2000). Release-independent short-term synaptic depression in cultured hippocampal neurons. *The Journal of Neuroscience* **20**(7): 2480-2494.

Brose, N. (2008). For better or for worse: Complexins regulate SNARE function and vesicle fusion. *Traffic* **9**(9): 1403-1413.

Brown, P. (2003). Oscillatory nature of human basal ganglia activity: Relationship to the pathophysiology of Parkinson's disease. *Movement Disorders* **18**(4): 357-363.

Bucher, D. and Goaillard, J.-M. (2011). Beyond faithful conduction: Short-term dynamics, neuromodulation, and long-term regulation of spike propagation in the axon. *Progress in Neurobiology* **94**(4): 307-346.

Bull, D. R., et al. (1990). Application of fast cyclic voltammetry to measurement of electrically evoked dopamine overflow from brain slices *in vitro*. *Journal of Neuroscience Methods* **32**(1): 37-44.

Bungay, P. M., et al. (2003). Microdialysis of dopamine interpreted with quantitative model incorporating probe implantation trauma. *Journal of Neurochemistry* **86**(4): 932-946.

Bunney, B. S., et al. (1973). Dopaminergic neurons: Effect of antipsychotic drugs and amphetamine on single cell activity. *Journal of Pharmacology and Experimental Therapeutics* **185**(3): 560-571.

Burré, J., et al. (2010). α-synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science* **329**(5999): 1663-1667.

Cabin, D. E., et al. (2002). Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha -synuclein. *The Journal of Neuroscience.* **22**(20): 8797-8807.

Cachope, R., et al. (2012). Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: Setting the tone for reward processing. *Cell Reports* **2**(1): 33-41.

Calabresi, P., et al. (1999). Activation of M1-like muscarinic receptors is required for the induction of corticostriatal LTP. *Neuropharmacology* **38**(2): 323-326.

Calabresi, P., et al. (2000). Synaptic transmission in the striatum: From plasticity to neurodegeneration. *Progress in Neurobiology* **61**(3): 231-265.

Calabresi, P., et al. (1998). Endogenous ACh enhances striatal NMDA-responses via M1-like muscarinic receptors and PKC activation. *European Journal of Neuroscience* **10**(9): 2887-2895.

Calabresi, P., et al. (2000). Acetylcholine-mediated modulation of striatal function. *Trends in Neurosciences* **23**(3): 120-126.

Calabresi, P., et al. (2007). Dopamine-mediated regulation of corticostriatal synaptic plasticity. *Trends in Neurosciences* **30**(5): 211-219.

Calabresi, P., et al. (1997). Abnormal synaptic plasticity in the striatum of mice lacking dopamine D2 receptors. *The Journal of Neuroscience* **17**(12): 4536-4544.

Capecchi, M. (1989). Altering the genome by homologous recombination. *Science* **244**(4910): 1288-1292.

Carboni, E., et al. (1989). Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. *Neuroscience* **28**(3): 653-661.

Cardozo, D. L. and 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.

Carlezon, W. A. J., et al. (1995). Habit-forming actions of nomifensine in nucleus accumbens. *Psychopharmacology* **122**(2): 194-197.

Carvelli, L., et al. (2008). Dopamine transporter/syntaxin 1A interactions regulate transporter channel activity and dopaminergic synaptic transmission. *Proceedings of the National Academy of Sciences* **105**(37): 14192-14197.

Carvelli, L., et al. (2004). Dopamine transporters depolarize neurons by a channel mechanism. *Proceedings of the National Academy of Sciences* **101**(45): 16046-16051.

Cass, W. A., et al. (1992). Differences in dopamine clearance and diffusion in rat striatum and nucleus accumbens following systemic cocaine administration. *Journal of Neurochemistry* **59**(1): 259-266.

Catterall, W. A. (2010). Signaling complexes of voltage-gated sodium and calcium channels. *Neuroscience Letters* **486**(2): 107-116.

Catterall, W. A. and A. P. Few (2008). Calcium channel regulation and presynaptic plasticity. *Neuron* **59**(6): 882-901.

Centonze, D., et al. (2003). Receptor subtypes involved in the presynaptic and postsynaptic actions of dopamine on striatal interneurons. *The Journal of Neuroscience* **23**(15): 6245-6254.

Chadchankar, H. and Yavich, L. (2011). Sub-regional differences and mechanisms of the short-term plasticity of dopamine overflow in striatum in mice lacking alpha-synuclein. *Brain Research* **1423**(0): 67-76.

Chan, A. W., et al. (2007). Munc18: A presynaptic transmitter release site N type (Cav2.2) calcium channel interacting protein. *Channels* **1**(1): 12-21.

Chan, C. S., et al. (2007). `Rejuvenation' protects neurons in mouse models of Parkinson's disease. *Nature* **447**(7148): 1081-1086.

Chandra, S., et al. (2005). [alpha]-synuclein cooperates with CSP[alpha] in preventing neurodegeneration. *Cell* **123**(3): 383-396.

Chapman, E. R. (2002). Synaptotagmin: A Ca2+ sensor that triggers exocytosis? *Nature Reviews Molecular Cell Biology* **3**(7): 498-508.

Charlety, P. J., et al. (1991). Burst firing of mesencephalic dopamine neurons is inhibited by somatodendritic application of kynurenate. *Acta Physiologica Scandinavica* **142**(1): 105-112.

Charlton, M. P., et al. (1982). Role of presynaptic calcium ions and channels in synaptic facilitation and depression at the squid giant synapse. *The Journal of Physiology* **323**(1): 173-193.

Chen, B. T. and Rice, M. E. (2001). Novel Ca2+ dependence and time course of somatodendritic dopamine release: Substantia nigra versus striatum. *The Journal of Neuroscience* **21**(19): 7841-7847.

Chen, L., et al. (2010). The mouse homeobox gene gbx2 is required for the development of cholinergic interneurons in the striatum. *The Journal of Neuroscience* **30**(44): 14824-14834.

Chen, R., et al. (2006). Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter. *Proceedings of the National Academy of Sciences* **103**(24): 9333-9338.

Cheng, H.-C., et al. (2010). Clinical progression in Parkinson's disease and the neurobiology of axons. *Annals of Neurology* **67**(6): 715-725.

Chéramy, A., et al. (1996). Cooperative contributions of cholinergic and NMDA receptors in the presynaptic control of dopamine release from synaptosomes of the rat striatum. *Journal of Pharmacology and Experimental Therapeutics* **276**(2): 616-625.

Chergui, K., et al. (1994). Nonlinear relationship between impulse flow, dopamine release and dopamine elimination in the rat brain *in vivo*. *Neuroscience* **62**(3): 641-645.

Chesselet, M. F. (1984). Presynaptic regulation of neurotransmitter release in the brain: Facts and hypothesis. *Neuroscience* **12**(2): 347-375.

Christoffersen, C. L. and Meltzer, L. T. (1995). Evidence for N-methyl-D-aspartate and ampa subtypes of the glutamate receptor on substantia nigra dopamine neurons: Possible preferential role for n-methyl-d-aspartate receptors. *Neuroscience* **67**(2): 373-381.

Ciliax, B., et al. (1995). The dopamine transporter: Immunochemical characterization and localization in brain. *The Journal of Neuroscience* **15**(3): 1714-1723.

Clements, M. A., et al. Cocaine enhances the contrast in phasic versus tonic dopamine signals via D2 auto- and heteroreceptors. *In preparation*.

Coizet, V., et al. (2006). Nociceptive responses of midbrain dopaminergic neurones are modulated by the superior colliculus in the rat. *Neuroscience* **139**(4): 1479-1493.

Comings, D. E., et al. (1996). Polygenic inheritance of tourette syndrome, stuttering, attention deficit hyperactivity, conduct, and oppositional defiant disorder: The additive and subtractive effect of the three dopaminergic genes--drd2, d beta h, and dat1. *American Journal of Medical Genetics* **67**(3): 264-288.

Comoli, E., et al. (2003). A direct projection from superior colliculus to substantia nigra for detecting salient visual events. *Nature Neuroscience* **6**(9): 974-980.

Congar, P., et al. (2002). D2 receptors inhibit the secretory process downstream from calcium influx in dopaminergic neurons: Implication of K+ channels. *Journal of Neurophysiology* **87**(2): 1046-1056.

Consolo, S., et al. (1992). Endogenous dopamine facilitates striatal in vivo acetylcholine release by acting on D1 receptors localized in the striatum. *Journal of Neurochemistry* **59**(4): 1555-1557.

Contant, C., et al. (1996). Ultrastructural characterization of the acetylcholine innervation in adult rat neostriatum. *Neuroscience* **71**(4): 937-947.

Cook, E. H. J., et al. (1995). Association of attention-deficit disorder and the dopamine transporter gene. *American Journal of Human Genetics* **56**(4): 993-998.

Cooke, J. D. and Quastel, D. M. J. (1973). The specific effect of potassium on transmitter release by motor nerve terminals and its inhibition by calcium. *The Journal of Physiology* **228**(2): 435-458.

Corti, O., et al. (2011). What genetics tells us about the causes and mechanisms of Parkinson's disease. *Physiological Reviews* **91**(4): 1161-1218.

Costa, R. M. (2007). Plastic corticostriatal circuits for action learning. *Annals of the New York Academy of Sciences* **1104**(1): 172-191.

Cragg, S. J. (2003). Variable dopamine release probability and short-term plasticity between functional domains of the primate striatum. *The Journal of Neuroscience.* **23**(10): 4378-4385.

Cragg, S. J. (2006). Meaningful silences: How dopamine listens to the ACh pause. *Trends in Neurosciences* **29**(3): 125-131.

Cragg, S. J., et al. (2000). Dopamine release and uptake dynamics within nonhuman primate striatum in vitro. *The Journal of Neuroscience* **20**(21): 8209-8217.

Cragg, S. J. and Rice, M. E. (2004). Dancing past the DAT at a DA synapse. *Trends in Neurosciences* **27**(5): 270-277.

Cubeddu, L. X. and Hoffmann, I. S. (1982). Operational characteristics of the inhibitory feedback mechanism for regulation of dopamine release via presynaptic receptors. *Journal of Pharmacology and Experimental Therapeutics* **223**(2): 497-501.

Daniel, J. A., et al. (2009). Functional heterogeneity at dopamine release sites. *The Journal of Neuroscience.* **29**(46): 14670-14680.

Dawson, T. M. and Dawson, V. L. (2003). Molecular pathways of neurodegeneration in Parkinson's disease. *Science* **302**(5646): 819-822.

Dawson, T. M., et al. (2010). Genetic animal models of Parkinson's disease. *Neuron* **66**(5): 646-661.

Day, J. J., et al. (2007). Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nature Neuroscience* **10**(8): 1020-1028.

de Rijk, M. C., et al. (2000). Prevalence of Parkinson's disease in europe: A collaborative study of population-based cohorts. Neurologic diseases in the elderly research group. *Neurology* **54**(11 Suppl 5): S21-23.

Debanne, D., et al. (1996). Paired-pulse facilitation and depression at unitary synapses in rat hippocampus: Quantal fluctuation affects subsequent release. *The Journal of Physiology* **491**(Pt 1): 163-176.

DeBoer, P. and Abercrombie, E. (1996). Physiological release of striatal acetylcholine *in vivo*: Modulation by D1 and D2 dopamine receptor subtypes. *Journal of Pharmacology and Experimental Therapeutics* **277**(2): 775-783.

del Campo, N., et al. (2011). The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biological Psychiatry* **69**(12): e145-e157.

del Castillo, J. and Katz, B. (1954). Statistical factors involved in neuromuscular facilitation and depression. *The Journal of Physiology* **124**(3): 574-585.

Delaney, K. and Tank, D. (1994). A quantitative measurement of the dependence of short-term synaptic enhancement on presynaptic residual calcium. *The Journal of Neuroscience* **14**(10): 5885-5902.

DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends in Neurosciences* **13**(7): 281-285.

Deng, P., et al. (2007). Involvement of Ih in dopamine modulation of tonic firing in striatal cholinergic interneurons. *The Journal of Neuroscience.* **27**(12): 3148-3156.

Descarries, L., et al. (1997). Diffuse transmission by acetylcholine in the CNS. *Progress in Neurobiology* **53**(5): 603-625.

Descarries, L. and Mechawar, N. (2000). Ultrastructural evidence for diffuse transmission by monoamine and acetylcholine neurons of the central nervous system. *Progress in brain research*. Agnati, Elsevier. **Volume 125:** 27-47.

Descarries, L., et al. (1996). Dual character, asynaptic and synaptic, of the dopamine innervation in adult rat neostriatum: A quantitative autoradiographic and immunocytochemical analysis. *Journal of Comparative Neurology* **375**(2): 167-186.

Ding, J. (2006). RGS4-dependent attenuation of M4 autoreceptor function in striatal cholinergic interneurons following dopamine depletion. *Nature*.

Ding, J., et al. (2006). RGS4-dependent attenuation of M4 autoreceptor function in striatal cholinergic interneurons following dopamine depletion. *Nature Neuroscience* **9**(6): 832-842.

Ding, J. B., et al. (2010). Thalamic gating of corticostriatal signaling by cholinergic interneurons. *Neuron* **67**(2): 294-307.

Dobrunz, L. E., et al. (1997). Very short-term plasticity in hippocampal synapses. *Proceedings of the National Academy of Sciences* **94**(26): 14843-14847.

Dobrunz, L. E. and Stevens, C. F. (1997). Heterogeneity of release probability, facilitation, and depletion at central synapses. *Neuron* **18**(6): 995-1008.

Dodson, P. D. and Forsythe, I. D. (2004). Presynaptic K+ channels: Electrifying regulators of synaptic terminal excitability. *Trends in Neurosciences* **27**(4): 210-217.

Dommett, E., et al. (2005). How visual stimuli activate dopaminergic neurons at short latency. *Science* **307**(5714): 1476-1479.

Dougherty, D. D., et al. (1999). Dopamine transporter density in patients with attention deficit hyperactivity disorder. *The Lancet* **354**(9196): 2132-2133.

Dunah, A. W. and Standaert, D. G. (2001). Dopamine D1 receptor-dependent trafficking of striatal nmda glutamate receptors to the postsynaptic membrane. *The Journal of Neuroscience* **21**(15): 5546-5558.

Egana, L. A., et al. (2009). Physical and functional interaction between the dopamine transporter and the synaptic vesicle protein synaptogyrin-3. *The Journal of Neuroscience.* **29**(14): 4592-4604.

Elmqvist, D. and Quastel, D. M. (1965). A quantitative study of end-plate potentials in isolated human muscle. *The Journal of Physiology* **178**(3): 505-529.

Eulitz, D., et al. (2007). Heterogeneous distribution of Kir3 potassium channel proteins within dopaminergic neurons in the mesencephalon of the rat brain. *Cellular and Molecular Neurobiology* **27**(3): 285-302.

Everitt, B. J. (2005). Neural systems of reinforcement for drug addiction: From actions to habits to compulsion. *Nature Neuroscience* **8**(11): 1481.

Everitt, B. J., et al. (2001). The neuropsychological basis of addictive behaviour. *Brain Research Reviews* **36**(2–3): 129-138.

Everitt, B. J. and Wolf, M. E. (2002). Psychomotor stimulant addiction: A neural systems perspective. *The Journal of Neuroscience* **22**(9): 3312-3320.

Ewing, A., et al. (1983). Direct *in vivo* monitoring of dopamine released from two striatal compartments in the rat. *Science* **221**(4606): 169-171.

Exley, R., et al. (2007). [alpha]6-containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine neurotransmission in nucleus accumbens. *Neuropsychopharmacology* **33**(9): 2158-2166.

Exley, R. and Cragg, S. J. (2008). Presynaptic nicotinic receptors: A dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. *British Journal of Pharmacology* **153**(S1): S283.

Fearnley, J. M. and Lees, A. J. (1991). Ageing and Parkinson's disease: Substantia nigra regional selectivity. *Brain* **Pt5**: 2283-2301.

Feng, J., et al. (2002). Regulation of neurotransmitter release by synapsin III. *The Journal of Neuroscience* **22**(11): 4372-4380.

Fernagut, P.-O. and Chesselet, M.-F. (2004). Alpha-synuclein and transgenic mouse models. *Neurobiology of Disease* **17**(2): 123-130.

Fioravante, D. and Regehr, W. G. (2011). Short-term forms of presynaptic plasticity. *Current Opinion in Neurobiology* **21**(2): 269-274.

Floresco, S. B., et al. (2003). Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nature Neuroscience* **6**(9): 968-973.

Ford, C. P., et al. (2010). Control of extracellular dopamine at dendrite and axon terminals. *The Journal of Neuroscience* **30**(20): 6975-6983.

Forsythe, I. D., et al. (1998). Inactivation of presynaptic calcium current contributes to synaptic depression at a fast central synapse. *Neuron* **20**(4): 797-807.

Fountaine, T. M., et al. (2008). The effect of α-synuclein knockdown on MPP+ toxicity in models of human neurons. *European Journal of Neuroscience* **28**(12): 2459-2473.

Freed, C., et al. (1995). Dopamine transporter immunoreactivity in rat brain. *Journal of Comparative Neurology* **359**(2): 340-349.

Gage, P. W. and Quastel, D. M. (1965). Dual effect of potassium on transmitter release. *Nature* **206**(984): 625-626.

Gainetdinov, R. R. and Caron, M. G. (2003). Monoamine transporters: From genes to behavior. *Annual Review of Pharmacology and Toxicology* **43**(1): 261-284.

Gainetdinov, R. R., et al. (1998). Re-evaluation of the role of the dopamine transporter in dopamine system homeostasis. *Brain Research Reviews* **26**(2–3): 148-153.

Galloway, M. (1990). Regulation of dopamine and serotonin synthesis by acute administration of cocaine. *Synapse* **6**(1): 63-72.

Garris, P., et al. (1994). Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. *The Journal of Neuroscience* **14**(10): 6084-6093.

Garris, P. A. and Rebec, G. V. (2002). Modeling fast dopamine neurotransmission in the nucleus accumbens during behavior. *Behavioural Brain Research* **137**(1-2): 47-63.

Garris, P. A., et al. (1997). Dopamine release and uptake rates both decrease in the partially denervated striatum in proportion to the loss of dopamine terminals. *Brain Research* **753**(2): 225-234.

Garris, P. A. and Wightman, R. M. (1995). DA release, uptake and diffusion. *Voltammetric methods in brain slices*. Boulton, A. A., Baker, G. B., and Adams, R. N. Humana**:** 179-212.

Geiger, J. R. P. and Jonas, P. (2000). Dynamic control of presynaptic Ca2+ inflow by fast-inactivating K+ channels in hippocampal mossy fiber boutons. *Neuron* **28**(3): 927-939.

Geisler, S., et al. (2007). Prominent activation of brainstem and pallidal afferents of the ventral tegmental area by cocaine. *Neuropsychopharmacology* **33**(11): 2688-2700.

Geppert, M., et al. (1997). The small GTP-binding protein rab3A regulates a late step in synaptic vesicle fusion. *Nature* **387**(6635): 810-814.

Gerfen, C. (1992). The neostriatal mosaic: Multiple levels of compartmental organization. *Trends in Neurosciences* **15**(4): 133-139.

Gerfen, C., et al. (1987). The neostriatal mosaic: Iii. Biochemical and developmental dissociation of patch-matrix mesostriatal systems. *The Journal of Neuroscience* **7**(12): 3935-3944.

Gerfen, C., et al. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* **250**(4986): 1429-1432.

Gerfen, C., et al. (1987). The neostriatal mosaic: Ii. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. *The Journal of Neuroscience* **7**(12): 3915-3934.

Gifford, A. N. and K. M. Johnson (1992). Comparison of the role of local anesthetic properties with dopamine uptake blockade in the inhibition of striatal and nucleus accumbens [3H]acetylcholine release by cocaine. *Journal of Pharmacology and Experimental Therapeutics* **263**(2): 757-761.

Giros, B., et al. (1996). Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* **379**(6566): 606-612.

Gispert, S., et al. (2003). Transgenic mice expressing mutant A53T human alpha-synuclein show neuronal dysfunction in the absence of aggregate formation. *Molecular and Cellular Neuroscience* **24**(2): 419-429.

Gitler, D., et al. (2004). Different presynaptic roles of synapsins at excitatory and inhibitory synapses. *The Journal of Neuroscience* **24**(50): 11368-11380.

Goldberg, J. A. and Reynolds, J. N. J. (2011). Spontaneous firing and evoked pauses in the tonically active cholinergic interneurons of the striatum. *Neuroscience* **198**(0): 27-43.

Goldberg, J. A. and Wilson, C. J. (2005). Control of spontaneous firing patterns by the selective coupling of calcium currents to calcium-activated potassium currents in striatal cholinergic interneurons. *The Journal of Neuroscience* **25**(44): 10230-10238.

Gonon, F. (1997). Prolonged and extrasynaptic excitatory action of dopamine mediated by D1 receptors in the rat striatum *in vivo*. *The Journal of Neuroscience* **17**(15): 5972-5978.

Grace, A. and Onn, S. (1989). Morphology and electrophysiological properties of immunocytochemically identified rat dopamine neurons recorded *in vitro*. *The Journal of Neuroscience* **9**(10): 3463-3481.

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

Grady, S., et al. (1992). Characterization of nicotinic receptor-mediated [3H]dopamine release from synaptosomes prepared from mouse striatum. *Journal of Neurochemistry* **59**(3): 848-856.

Graham, D. R. and Sidhu, A. (2010). Mice expressing the A53T mutant form of human alpha-synuclein exhibit hyperactivity and reduced anxiety-like behavior. *Journal of Neuroscience Research* **88**(8): 1777-1783.

Grant, S., et al. (2000). Drug abusers show impaired performance in a laboratory test of decision making. *Neuropsychologia* **38**(8): 1180-1187.

Graveland, G. A. and Difiglia, M. (1985). The frequency and distribution of medium-sized neurons with indented nuclei in the primate and rodent neostriatum. *Brain Research* **327**(1–2): 307-311.

Graveland, G. A., et al. (1985). A golgi study of the human neostriatum: Neurons and afferent fibers. *Journal of Comparative Neurology* **234**(3): 317-333.

Gray, R., et al. (1996). Hippocampal synaptic transmission enhanced by low concentrations of nicotine. *Nature* **383**(6602): 713-716.

Graybiel, A.,M. et al. (1994). The basal ganglia and adaptive motor control. *Science* **265**(5180): 1826-1831.

Graybiel, A. M. (2008). Habits, rituals, and the evaluative brain. *Annual Review of Neuroscience* **31**(1): 359-387.

Greif, G., et al. (1995). Dopamine-modulated potassium channels on rat striatal neurons: Specific activation and cellular expression. *The Journal of Neuroscience* **15**(6): 4533-4544.

Grilli, M., et al. (2012). *In vitro* exposure to nicotine induces endocytosis of presynaptic ampa receptors modulating dopamine release in rat nucleus accumbens nerve terminals. *Neuropharmacology* **63**(5): 916-926.

Gu, H., et al. (1994). Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence. *Journal of Biological Chemistry* **269**(10): 7124-7130.

Gubernator, N. G., et al. (2009). Fluorescent false neurotransmitters visualize dopamine release from individual presynaptic terminals. *Science* **324**(5933): 1441-1444.

Guiramand, J., et al. (1995). Alternative splicing of the dopamine D2 receptor directs specificity of coupling to g-proteins. *Journal of Biological Chemistry* **270**(13): 7354-7358.

Gunaydin, L. A., et al. (2010). Ultrafast optogenetic control. *Nature Neuroscience* **13**(3): 387-392.

Haber, S. N. (2000). Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *The Journal of Neuroscience* **20**(6): 2369.

Haber, S. N. (2003). The primate basal ganglia: Parallel and integrative networks. *Journal of Chemical Neuroanatomy* **26**(4): 317-330.

Haber, S. N., et al. (2006). Reward-related cortical inputs define a large striatal region in primates that interface with associative cortical connections, providing a substrate for incentive-based learning. *The Journal of Neuroscience* **26**(32): 8368-8376.

He, Y., et al. (2002). Contribution of presynaptic Na+ channel inactivation to paired-pulse synaptic depression in cultured hippocampal neurons. *Journal of Neurophysiology* **87**(2): 925-936.

Hernández-Echeagaray, E., et al. (2004). Modulation of ampa currents by D2 dopamine receptors in striatal medium-sized spiny neurons: Are dendrites necessary? *European Journal of Neuroscience* **19**(9): 2455-2463.

Hernández-López, S., et al. (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+ currents and excitability via a novel PLCβ1–IP3–calcineurin-signaling cascade. *The Journal of Neuroscience* **20**(24): 8987-8995.

Hersch, S., et al. (1994). Distribution of M1-M4 muscarinic receptor proteins in the rat striatum: Light and electron microscopic immunocytochemistry using subtype- specific antibodies. *The Journal of Neuroscience* **14**(5): 3351-3363.

Hervé, D., et al. (1987). Serotonin axon terminals in the ventral tegmental area of the rat: Fine structure and synaptic input to dopaminergic neurons. *Brain Research* **435**(1–2): 71-83.

Hill, E. R., et al. (2009). Potencies of cocaine methiodide on major cocaine targets in mice. *PLoS ONE* **4**(10): e7578.

Hill, J., et al. (1993). Immunocytochemical localization of a neuronal nicotinic receptor: The beta 2-subunit. *The Journal of Neuroscience* **13**(4): 1551-1568.

Hollerman, J. R. and Schultz, W. (1998). Dopamine neurons report an error in the temporal prediction of reward during learning. *Nature Neuroscience* **1**(4): 304-309.

Hoover, D. B., et al. (1978). A mapping of the distribution of acetylcholine, choline acetyltransferase and acetylcholinesterase in discrete areas of rat brain. *Brain Research* **153**(2): 295-306.

Hornykiewicz, O. (1998). Biochemical aspects of Parkinson's disease. *Neurology* **51**.

Howe, A. and Surmeier, D. J. (1995). Muscarinic receptors modulate N-, P-, and L-type Ca2+ currents in rat striatal neurons through parallel pathways. *The Journal of Neuroscience* **15**(1): 458-469.

Hu, G., et al. (1999). The regulation of dopamine transmission by metabotropic glutamate receptors. *Journal of Pharmacology and Experimental Therapeutics* **289**(1): 412-416.

Huang, X., et al. (2009). Mechanism for cocaine blocking the transport of dopamine: Insights from molecular modeling and dynamics simulations. *The Journal of Physical Chemistry B* **113**(45): 15057-15066.

Hyland, B. I., et al. (2002). Firing modes of midbrain dopamine cells in the freely moving rat. *Neuroscience* **114**(2): 475-492.

Imai, Y. and Lu, B. (2011). Mitochondrial dynamics and mitophagy in Parkinson's disease: Disordered cellular power plant becomes a big deal in a major movement disorder. *Current Opinion in Neurobiology* **21**(6): 935-941.

Ingram, S. L., et al. (2002). Dopamine transporter-mediated conductances increase excitability of midbrain dopamine neurons. *Nature Neuroscience* **5**(10): 971-978.

Ito, M. and K. Doya (2011). Multiple representations and algorithms for reinforcement learning in the cortico-basal ganglia circuit. *Current Opinion in Neurobiology* **21**(3): 368-373.

Jae Kim, S., et al. (2006). α-synuclein enhances dopamine D2 receptor signaling. *Brain Research* **1124**(1): 5-9.

Javoy, F. and Glowinski, J. (1971). Dynamic characteristic of the 'functional compartment' of dopamine in dopaminergic terminals of the rat striatum. *Journal of Neurochemistry* **18**(7): 1305-1311.

Jeon, J., et al. (2010). A subpopulation of neuronal M4 muscarinic acetylcholine receptors plays a critical role in modulating dopamine-dependent behaviors. *The Journal of Neuroscience* **30**(6): 2396-2405.

Joel, D. and Weiner , I. (1997). The connections of the primate subthalamic nucleus: Indirect pathways and the open-interconnected scheme of basal ganglia-thalamocortical circuitry. *Brain Research Reviews* **23**(1–2): 62-78.

John, C. E. and Jones , S. R. (2007). Voltammetric characterization of the effect of monoamine uptake inhibitors and releasers on dopamine and serotonin uptake in mouse caudate-putamen and substantia nigra slices. *Neuropharmacology* **52**(8): 1596-1605.

Johnson, S., et al. (1992). Burst firing in dopamine neurons induced by n-methyl-d-aspartate: Role of electrogenic sodium pump. *Science* **258**(5082): 665-667.

Johnson, S. W. and Seutin, V. (1997). Bicuculline methiodide potentiates nmda-dependent burst firing in rat dopamine neurons by blocking apamin-sensitive Ca2+-activated K+ currents. *Neuroscience Letters* **231**(1): 13-16.

Jones, I. W., et al. (2001). Presynaptic localisation of the nicotinic acetylcholine receptor β2 subunit immunoreactivity in rat nigrostriatal dopaminergic neurones. *The Journal of Comparative Neurology* **439**(2): 235-247.

Jones, S. R., et al. (1998). Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. *The Journal of Neuroscience* **18**(6): 1979-1986.

Jones, S. R., et al. (1995). Comparison of dopamine uptake in the basolateral amygdaloid nucleus, caudate-putamen, and nucleus accumbens of the rat. *Journal of Neurochemistry* **64**(6): 2581-2589.

Jones, S. R., et al. (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): 396-403.

Jones SR, et al. (1997). Profound neuronal plasticity in response to inactivation of the dopamine transporter. *Proceedings of the National Academy of Sciences* **95**(7): 4029-4034.

Jones, S. R., et al. (1996). Functional and anatomical evidence for different dopamine dynamics in the core and shell of the nucleus accumbens in slices of rat brain. *Synapse* **23**(3): 224-231.

Joshua, M., et al. (2008). Midbrain dopaminergic neurons and striatal cholinergic interneurons encode the difference between reward and aversive events at different epochs of probabilistic classical conditioning trials. *The Journal of Neuroscience* **28**(45): 11673-11684.

Kang, Y. and Kitai, S. T. (1993). Calcium spike underlying rhythmic firing in dopaminergic neurons of the rat substantia nigra. *Neuroscience Research* **18**(3): 195-207.

Kapatos, G. and Zigmond , M. J. (1979). Effect of haloperidol on dopamine synthesis and tyrosine hydroxylase in striatal synaptosomes. *Journal of Pharmacology and Experimental Therapeutics* **208**(3): 468-475.

Kása, P., et al. (1997). The cholinergic system in Alzheimer's disease. *Progress in Neurobiology* **52**(6): 511-535.

Kasanetz, F., et al. (2010). Transition to addiction is associated with a persistent impairment in synaptic plasticity. *Science* **328**(5986): 1709-1712.

Katz, B. and Miledi, R. (1967). Ionic requirements of synaptic transmitter release. *Nature* **215**(5101): 651-651.

Katz, B. and Miledi, R. (1968). The role of calcium in neuromuscular facilitation. *The Journal of Physiology* **195**(2): 481-492.

Kawagoe, R., et al. (2004). Reward-predicting activity of dopamine and caudate neurons—a possible mechanism of motivational control of saccadic eye movement. *Journal of Neurophysiology* **91**(2): 1013-1024.

Kawaguchi, Y. (1992). Large aspiny cells in the matrix of the rat neostriatum in vitro: Physiological identification, relation to the compartments and excitatory postsynaptic currents. *Journal of Neurophysiology* **67**(6): 1669-1682.

Kawaguchi, Y. (1993). Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. *The Journal of Neuroscience* **13**(11): 4908-4923.

Kawaguchi, Y., et al. (1990). Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. *The Journal of Neuroscience* **10**(10): 3421-3438.

Kawaguchi, Y., et al. (1995). Striatal interneurones: Chemical, physiological and morphological characterization. *Trends in Neurosciences* **18**(12): 527-535.

Kelly, R. S. and Wightman, R. M. (1987). Detection of dopamine overflow and diffusion with voltammetry in slices of rat brain. *Brain Research* **423**(1–2): 79-87.

Kemp, J. M. and Powell, T. P. S. (1971). The structure of the caudate nucleus of the cat: Light and electron microscopy. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences* **262**(845): 383-401.

Kennedy, R., et al. (1992). Dynamic observation of dopamine autoreceptor effects in rat striatal slices. *Journal of Neurochemistry* **59**(2): 449-455.

Khaliq, Z. M. and Bean, B. P. (2010). Pacemaking in dopaminergic ventral tegmental area neurons: Depolarizing drive from background and voltage-dependent sodium conductances. *The Journal of Neuroscience* **30**(21): 7401-7413.

Khanna, R., et al. (2007). A proteomic screen for presynaptic terminal N-type calcium channel (Cav2.2) binding partners *Journal of Biochemistry and Molecular Biology* **40**(3): 302-314.

Kile, B. M., et al. (2010). Synapsins differentially control dopamine and serotonin release. *The Journal of Neuroscience* **30**(29): 9762-9770.

Kincaid, A. E., et al. (1991). Evidence for a projection from the globus pallidus to the entopeduncular nucleus in the rat. *Neuroscience Letters* **128**(1): 121-125.

Kitai, S. T. and Deniau, J. M. (1981). Cortical inputs to the subthalamus: Intracellular analysis. *Brain Research* **214**(2): 411-415.

Kitai, S. T. and Surmeier, D. J. (1993). Cholinergic and dopaminergic modulation of potassium conductances in neostriatal neurons. *Advances in Neurology* **60**: 40-52.

Kiyatkin, E. A. and Rebec, G. V. (1998). Heterogeneity of ventral tegmental area neurons: Single-unit recording and iontophoresis in awake, unrestrained rats. *Neuroscience* **85**(4): 1285-1309.

Knowlton, B. J., et al. (1996). A neostriatal habit learning system in humans. *Science* **273**(5280): 1399-1402.

Kolokotroni, K., et al. (2011). Acute nicotine increases both impulsive choice and behavioural disinhibition in rats. *Psychopharmacology* **217**(4): 455-473.

Koob, G. F. (1992). Neural mechanisms of drug reinforcementa. *Annals of the New York Academy of Sciences* **654**(1): 171-191.

Koos, T. and Tepper, J. M. (1999). Inhibitory control of neostriatal projection neurons by gabaergic interneurons. *Nature Neuroscience* **2**(5): 467-472.

Koós, T. and Tepper, J. M. (2002). Dual cholinergic control of fast-spiking interneurons in the neostriatum. *The Journal of Neuroscience* **22**(2): 529-535.

Kourrich, S., et al. (2007). Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. *The Journal of Neuroscience* **27**(30): 7921-7928.

Koushika, S. P., et al. (2001). A post-docking role for active zone protein RIM. *Nature Neuroscience* **4**(10): 997-1005.

Kreitzer, A. C. and Malenka, R. C. (2005). Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. *The Journal of Neuroscience* **25**(45): 10537-10545.

Kreitzer, A. C. and Malenka, R. C. (2008). Striatal plasticity and basal ganglia circuit function. *Neuron* **60**(4): 543-554.

Kreuger, B. K. (1990). Kinetics and block of dopamine uptake in synaptosomes from rat caudate nucleus. *Journal of Neurochemistry* **55**(1): 260-267.

Krüger, R., et al. (1998). Ala30Pro mutation in the gene encoding α-synuclein in parkinson's disease. *Nature Genetics* **18**(2): 106-108.

Kuczenski, R. and Sega, D. S. (1992). Differential effects of amphetamine and dopamine uptake blockers (cocaine, nomifensine) on caudate and accumbens dialysate dopamine and 3-methoxytyramine. *Journal of Pharmacology and Experimental Therapeutics* **262**(3): 1085-1094.

Kuhr, W. G. and Wightman, R. M. (1986). Real-time measurement of dopamine release in rat brain. *Brain Research* **381**(1): 168-171.

Kurz, A., et al. (2010). A53T-alpha-synuclein overexpression impairs dopamine signaling and striatal synaptic plasticity in old mice. *PLoS ONE* **5**(7): e11464.

Kurz, A., et al. (2011). A53T-alpha-synuclein-overexpression in the mouse nigrostriatal pathway leads to early increase of 14-3-3 epsilon and late increase of GFAP. *Journal of Neural Transmission*: 1-16.

Lapper, S. R. and J. P. Bolam (1992). Input from the frontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat. *Neuroscience* **51**(3): 533-545.

Larsen, K. E., et al. (2006). {alpha}-synuclein overexpression in PC12 and chromaffin cells impairs catecholamine release by interfering with a late step in exocytosis. *The Journal of Neuroscience.* **26**(46): 11915-11922.

Le Moine, C. and Bloch, B. (1995). D1 and D2 dopamine receptor gene expression in the rat striatum: Sensitive crna probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. *The Journal of Comparative Neurology* **355**(3): 418-426.

Le Moine, C., et al. (1991). Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. *Proceedings of the National Academy of Sciences* **88**(10): 4205-4209.

Le Moine, C., et al. (1990). D2 dopamine receptor gene expression by cholinergic neurons in the rat striatum. *Neuroscience Letters* **117**(3): 248-252.

Lee, F. J. S., et al. (2001). Direct binding and functional coupling of {alpha}-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. *FASEB J.* **15**(6): 916-926.

Lee, I. H., et al. (2006). Activity of tonically active neurons in the monkey putamen during initiation and withholding of movement. *Journal of Neurophysiology* **95**(4): 2391-2403.

Lee, K.-H., et al. (2004). Syntaxin 1A and receptor for activated C kinase interact with the N-terminal region of human dopamine transporter. *Neurochemical Research* **29**(7): 1405-1409.

Lee, T., et al. (2001). Differential time-course profiles of dopamine release and uptake changes induced by three dopamine uptake inhibitors. *Synapse* **41**(4): 301-310.

Lehmann, J. and Langer, S. Z. (1982). Muscarinic receptors on dopamine terminals in the cat caudate nucleus: Neuromodulation of [3H]dopamine release in vitro by endogenous acetylcholine. *Brain Research* **248**(1): 61-69.

Lester, J., et al. (1993). Colocalization of D1 and D2 dopamine receptor mRNAs in striatal neurons. *Brain Research* **621**(1): 106-110.

Levey, A. I., et al. (1993). Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. *Proceedings of the National Academy of Sciences* **90**(19): 8861-8865.

Li, L., et al. (1995). Impairment of synaptic vesicle clustering and of synaptic transmission, and increased seizure propensity, in synapsin I-deficient mice. *Proceedings of the National Academy of Sciences* **92**(20): 9235-9239.

Lindgren, N., et al. (2001). Dopamine D2 receptors regulate tyrosine hydroxylase activity and phosphorylation at ser40 in rat striatum. *European Journal of Neuroscience* **13**(4): 773-780.

Liss, B., et al. (2001). Tuning pacemaker frequency of individual dopaminergic neurons by Kv4.3L and KChip3.1 transcription. *EMBO J* **20**(20): 5715-5724.

Liss, B., et al. (2005). K-ATP channels promote the differential degeneration of dopaminergic midbrain neurons. *Nature Neuroscience* **8**(12): 1742-1751.

Liss, B. and Roeper, J. (2008). Individual dopamine midbrain neurons: Functional diversity and flexibility in health and disease. *Brain Research Reviews* **58**(2): 314-321.

Ljungberg, T., et al. (1992). Responses of monkey dopamine neurons during learning of behavioral reactions. *Journal of Neurophysiology* **67**(1): 145-163.

Lovinger, D. M. (2010). Neurotransmitter roles in synaptic modulation, plasticity and learning in the dorsal striatum. *Neuropharmacology* **58**(7): 951-961.

Lundblad, M., et al. (2012). Impaired neurotransmission caused by overexpression of α-synuclein in nigral dopamine neurons. *Proceedings of the National Academy of Sciences* **109**(9): 3213-3219.

Maiya, R., et al. (2007). Defining the dopamine transporter proteome by convergent biochemical and in silico analyses. *Genes, Brain and Behavior* **6**(1): 97-106.

Mallet, N., et al. (2012). Dichotomous organization of the external globus pallidus. *Neuron* **74**(6): 1075-1086.

Mameli-Engvall, M., et al. (2006). Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. *Neuron* **50**(6): 911-921.

Manabe, T., et al. (1993). Modulation of synaptic transmission and long-term potentiation: Effects on paired pulse facilitation and epsc variance in the CA1 region of the hippocampus. *Journal of Neurophysiology* **70**(4): 1451-1459.

Mansvelder, H. D., et al. (2002). Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. *Neuron* **33**(6): 905-919.

Mansvelder, H. D. and McGehee, D. S. (2000). Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron* **27**(2): 349-357.

Markram, H., et al. (1998). Differential signaling via the same axon of neocortical pyramidal neurons. *Proceedings of the National Academy of Sciences* **95**(9): 5323-5328.

Maroteaux, L., et al. (1988). Synuclein: A neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *The Journal of Neuroscience* **8**(8): 2804-2815.

Marshall, D., et al. (1997). Tetrodotoxin-sensitivity of nicotine-evoked dopamine release from rat striatum. *Neuropharmacology* **35**(11): 1531-1536.

Marshall, J. F., et al. (1990). Dopamine high-affinity transport site topography in rat brain: Major differences between dorsal and ventral striatum. *Neuroscience* **37**(1): 11-21.

Martel, P., et al. (2011). Role of Kv1 potassium channels in regulating dopamine release and presynaptic D2 receptor function. *PLoS ONE* **6**(5): e20402.

Matsuda, W. (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *The Journal of Neuroscience* **29**(2): 444.

Matsumoto, M. and Hikosaka, O. (2007). Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature* **447**(7148): 1111-1115.

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

Matsumoto, N., et al. (1999). Nigrostriatal dopamine system in learning to perform sequential motor tasks in a predictive manner. *Journal of Neurophysiology* **82**(2): 978-998.

Matsumoto, N., et al. (2001). Neurons in the thalamic CM-Pf complex supply striatal neurons with information about behaviorally significant sensory events. *Journal of Neurophysiology* **85**(2): 960-976.

Maurice, N., et al. (2004). D2 dopamine receptor-mediated modulation of voltage-dependent na+ channels reduces autonomous activity in striatal cholinergic interneurons. *The Journal of Neuroscience.* **24**(46): 10289-10301.

Mayer, A., et al. (1988). Transmitter release patterns of noradrenergic, dopaminergic and cholinergic axons in rabbit brain slices during short pulse trains, and the operation of presynaptic autoreceptors. *Naunyn-Schmiedeberg's Archives of Pharmacology* **338**(6): 632-643.

McKay, B. E., et al. (2007). Regulation of synaptic transmission and plasticity by neuronal nicotinic acetylcholine receptors. *Biochemical Pharmacology* **74**(8): 1120-1133.

Meador-Woodruff, J. H., et al. (1991). Comparison of the distributions of D1 and D2 dopamine receptor mRNAs in rat brain. *Neuropsychopharmacology* **5**(4): 231-242.

Meisenzahl, E. M., et al. (2007). The role of dopamine for the pathophysiology of schizophrenia. *International Review of Psychiatry* **19**(4): 337-345.

Mendez, I., et al. Effects of acute administration of nicotinic and muscarinic cholinergic agonists and antagonists on performance in different cost–benefit decision making tasks in rats. *Psychopharmacology*: 1-11.

Mercuri, N. B., et al. (1997). Loss of autoreceptor function in dopaminergic neurons from dopamine d2 receptor deficient mice. *Neuroscience* **79**(2): 323-327.

Michael, D. J. and Wightman, R. M. (1999). Electrochemical monitoring of biogenic amine neurotransmission in real time. *Journal of Pharmaceutical and Biomedical Analysis* **19**(1–2): 33-46.

Mirenowicz, J. and Schultz, W. (1996). Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature* **379**(6564): 449-451.

Mitchell, M., et al. (2011). Effects of acute administration of nicotine, amphetamine, diazepam, morphine, and ethanol on risky decision-making in rats. *Psychopharmacology* **218**(4): 703-712.

Mochida, S., et al. (2008). Regulation of presynaptic Cav2.1 channels by Ca2+ sensor proteins mediates short-term synaptic plasticity. *Neuron* **57**(2): 210-216.

Monakow, K. H.-v., et al. (1978). Projections of the precentral motor cortex and other cortical areas of the frontal lobe to the subthalamic nucleus in the monkey. *Experimental Brain Research* **33**(3): 395-403.

Montague, P. R., et al. (2004). Dynamic gain control of dopamine delivery in freely moving animals. *The Journal of Neuroscience.* **24**(7): 1754-1759.

Morris, G., et al. (2004). Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. *Neuron* **43**(1): 133-143.

Mouse-Genome-Sequencing-Consortium (2002). Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**(6915): 520-562.

Muñoz-Cuevas, J., et al. (2004). Characterization of release-independent short-term depression in the juvenile rat hippocampus. *The Journal of Physiology* **558**(2): 527-548.

Murphy, D. D., et al. (2000). Synucleins are developmentally expressed, and alpha -synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *The Journal of Neuroscience.* **20**(9): 3214-3220.

Nagel, G., et al. (2005). Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses. *Current Biology* **15**(24): 2279-2284.

Nagel, G., et al. (2003). Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proceedings of the National Academy of Sciences* **100**(24): 13940-13945.

Nakahara, H., et al. (2004). Dopamine neurons can represent context-dependent prediction error. *Neuron* **41**(2): 269-280.

Nemani, V. M., et al. (2010). Increased expression of α-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. *Neuron* **65**(1): 66-79.

Nestler, E. J. (2001). Molecular basis of long-term plasticity underlying addiction. *Nature Reviews Neuroscience* **2**(2): 119-128.

Nirenberg, M., et al. (1996). The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. *The Journal of Neuroscience* **16**(2): 436-447.

Nirenberg, M. J., et al. (1997). The dopamine transporter: Comparative ultrastructure of dopaminergic axons in limbic and motor compartments of the nucleus accumbens. *The Journal of Neuroscience* **17**(18): 6899-6907.

Niznik, H. and Van Tol, H. H. (1992). Dopamine receptor genes: New tools for molecular psychiatry. *Journal of Psychiatry and Neuroscience* **17**(4): 158-180.

O'Sullivan, S. S., et al. (2008). Nonmotor symptoms as presenting complaints in Parkinson's disease: A clinicopathological study. *Movement Disorders* **23**(1): 101-106.

Olanow, C. W. and Tatton, W. G. (1999). Etiology and pathogenesis of Parkinson's disease. *Annual Review of Neuroscience* **22**(1): 123-144.

Oldenburg, I. A. and Ding, J. B. (2011). Cholinergic modulation of synaptic integration and dendritic excitability in the striatum. *Current Opinion in Neurobiology* **21**(3): 425-432.

Olds, J. and Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of Comparative and Physiological Pscychology* **47**(6): 419-427.

Olds, M. E. (1982). Reinforcing effects of morphine in the nucleus accumbens. *Brain Research* **237**(2): 429-440.

Oswald, M. J., et al. (2009). Ih current generates the afterhyperpolarisation following activation of subthreshold cortical synaptic inputs to striatal cholinergic interneurons. *The Journal of Physiology* **587**(24): 5879-5897.

Ouardouz, M. and Lacaille, J.-C. (1997). Properties of unitary IPSCs in hippocampal pyramidal cells originating from different types of interneurons in young rats. *Journal of Neurophysiology* **77**(4): 1939-1949.

Overton, P. and Clark, D. (1992). Iontophoretically administered drugs acting at theN-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat. *Synapse* **10**(2): 131-140.

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

Pakhotin, P. and Bracci, E. (2007). Cholinergic interneurons control the excitatory input to the striatum. *The Journal of Neuroscience* **27**(2): 391-400.

Paladini, C. A., et al. (1999). GABAA receptor stimulation blocks NMDA-induced bursting of dopaminergic neurons in vitro by decreasing input resistance. *Brain Research* **832**(1–2): 145-151.

Palij, P., et al. (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.

Park, S. M., et al. (2002). Evidence that α-synuclein functions as a negative regulator of ca++-dependent α-granule release from human platelets. *Blood* **100**(7): 2506-2514.

Percheron, G. and Filion, M. (1991). Parallel processing in the basal ganglia: Up to a point. *Trends in Neurosciences* **14**(2): 55-56.

Perez-Rosello, T., et al. (2005). Cholinergic control of firing pattern and neurotransmission in rat neostriatal projection neurons: Role of Cav2.1 and Cav2.2 Ca2+ channels. *Journal of Neurophysiology* **93**(5): 2507-2519.

Perez, R. G., et al. (2002). A role for α-synuclein in the regulation of dopamine biosynthesis. *The Journal of Neuroscience* **22**(8): 3090-3099.

Phelps, P., et al. (1989). The generation and differentiation of cholinergic neurons in rat caudate-putamen. *Brain Research. Developmental Brain Research* **46**(1): 47-60.

Phillips, G. D., et al. (1994). Bilateral intra-accumbens self-administration of d-amphetamine: Antagonism with intra-accumbens SCH-23390 and sulpiride. *Psychopharmacology* **114**(3): 477-485.

Phillips, P. E. M., et al. (2002). Time window of autoreceptor-mediated inhibition of limbic and striatal dopamine release. *Synapse* **44**(1): 15-22.

Pickel, V. M., et al. (1981). Ultrastructural immunocytochemical localization of tyrosine hydroxylase in the neostriatum. *Brain Research* **225**(2): 373-385.

Pickel, V. M., et al. (1975). Immunohistochemical localization of tyrosine hydroxylase in brain by light and electron microscopy. *Brain Research* **85**(2): 295-300.

Pickel, V. M., et al. (1996). Ultrastructural view of central catecholaminergic transmission: Immunocytochemical localization of synthesizing enzymes, transporters and receptors. *Journal of Neurocytology* **25**(12): 843-856.

Pidoplichko, V. I., et al. (1997). Nicotine activates and desensitizes midbrain dopamine neurons. *Nature* **390**(6658): 401-404.

Pidoplichko, V. I., et al. (2004). Nicotinic cholinergic synaptic mechanisms in the ventral tegmental area contribute to nicotine addiction. *Learning & Memory* **11**(1): 60-69.

Pieribone, V. A., et al. (2002). Expression of synapsin III in nerve terminals and neurogenic regions of the adult brain. *The Journal of Comparative Neurology* **454**(2): 105-114.

Pieribone, V. A., et al. (1995). Distinct pools of synaptic vesicles in neurotransmitter release. *Nature* **375**(6531): 493-497.

Pisani, A., et al. (2000). Activation of D2-like dopamine receptors reduces synaptic inputs to striatal cholinergic interneurons. *The Journal of Neuroscience.* **20**(7): 69.

Polymeropoulos, M. H., et al. (1997). Mutation in the {alpha}-synuclein gene identified in families with Parkinson's disease. *Science* **276**(5321): 2045-2047.

Porter, J. N., et al. (2011). Chronic cocaine self-administration in rhesus monkeys: Impact on associative learning, cognitive control, and working memory. *The Journal of Neuroscience* **31**(13): 4926-4934.

Porton, B., et al. (2010). Mice lacking synapsin III show abnormalities in explicit memory and conditioned fear. *Genes, Brain and Behavior* **9**(3): 257-268.

Pothos, E. N., et al. (1998). Presynaptic recording of quanta from midbrain dopamine neurons and modulation of the quantal size. *The Journal of Neuroscience.* **18**(11): 4106-4118.

Povlock, S. L. and Schenk, J. O. (1997). A multisubstrate kinetic mechanism of dopamine transport in the nucleus accumbens and its inhibition by cocaine. *Journal of Neurochemistry* **69**(3): 1093-1105.

Rajakumar, N., et al. (1994). The pallidostriatal projection in the rat: A recurrent inhibitory loop? *Brain Research* **651**(1–2): 332-336.

Rapier, C., et al. (1988). Stereoselective nicotine-induced release of dopamine from striatal synaptosomes: Concentration dependence and repetitive stimulation. *Journal of Neurochemistry* **50**(4): 1123-1130.

Ravel, S., et al. (1999). Tonically active neurons in the monkey striatum do not preferentially respond to appetitive stimuli. *Experimental Brain Research* **128**(4): 531-534.

Ravel, S., et al. (2003). Responses of tonically active neurons in the monkey striatum discriminate between motivationally opposing stimuli. *The Journal of Neuroscience* **23**(24): 8489-8497.

Raz, A., et al. (1996). Neuronal synchronization of tonically active neurons in the striatum of normal and parkinsonian primates. *Journal of Neurophysiology* **76**(3): 2083-2088.

Redgrave, P., et al. (2008). What is reinforced by phasic dopamine signals? *Brain Research Reviews* **58**(2): 322-339.

Regehr, W., et al. (1994). The role of presynaptic calcium in short-term enhancement at the hippocampal mossy fiber synapse. *The Journal of Neuroscience* **14**(2): 523-537.

Reith, M. E. A., et al. (1985). Locomotor effects of cocaine, cocaine congeners, and local anesthetics in mice. *Pharmcology, Biochemistry and Behaviour* **23**(5): 831-836.

Reynolds, J. N. J., et al. (2004). Modulation of an afterhyperpolarization by the substantia nigra induces pauses in the tonic firing of striatal cholinergic interneurons. *The Journal of Neuroscience* **24**(44): 9870-9877.

Rice, M. E. and Cragg, S. J. (2004). Nicotine amplifies reward-related dopamine signals in striatum. *Nature Neuroscience* **7**(6): 583.

Rice, M. E., et al. (1985). Simultaneous voltammetric and chemical monitoring of dopamine release in situ. *Brain Research* **340**(1): 151-155.

Richardson, N. R. and Gratton , A. (1996). Behavior-relevant changes in nucleus accumbens dopamine transmission elicited by food reinforcement: An electrochemical study in rat. *The Journal of Neuroscience* **16**(24): 8160-8169.

Richfield, E., et al. (1989). Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. *Neuroscience* **30**(3): 767-777.

Ritz, M., et al. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* **237**(4819): 1219-1223.

Ritz, M. C., et al. (1990). Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: A structure-activity study. *Life Sciences* **46**(9): 635-645.

Rizzoli, S. O. and Betz, W. J. (2005). Synaptic vesicle pools. *Nature Reviews Neuroscience* **6**(1): 57-69.

Roberts, D. C., et al. (1977). On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmcology, Biochemistry and Behaviour* **6**(6): 615-620.

Robinson, D. L. and Wightman, R. M. (2004). Nomifensine amplifies subsecond dopamine signals in the ventral striatum of freely-moving rats. *Journal of Neurochemistry* **90**(4): 894-903.

Robinson, D. L. and Wightman, R. M. (2007). Rapid dopamine release in freely moving rats. *Electrochemical methods for neuroscience.* B. L. Michael AC. Boca Raton CRC Press.

Roitman, M. F., et al. (2004). Dopamine operates as a subsecond modulator of food seeking. *The Journal of Neuroscience* **24**(6): 1265-1271.

Royer, S., et al. (2000). Switching off and on of synaptic sites ataplysia sensorimotor synapses. *The Journal of Neuroscience* **20**(2): 626-638.

Saal, D., et al. (2003). Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron* **37**(4): 577-582.

Sabatini, B. L. and Regehr, W. G. (1996). Timing of neurotransmission at fast synapses in the mammalian brain. *Nature* **384**(6605): 170-172.

Sakrikar, D., et al. (2012). Attention deficit/hyperactivity disorder-derived coding variation in the dopamine transporter disrupts microdomain targeting and trafficking regulation. *The Journal of Neuroscience* **32**(16): 5385-5397.

Salamone, J. D. (1996). The behavioral neurochemistry of motivation: Methodological and conceptual issues in studies of the dynamic activity of nucleus accumbens dopamine. *Journal of Neuroscience Methods* **64**(2): 137-149.

Samii, A., et al. (2004). Parkinson's disease. *The Lancet* **363**(9423): 1783-1793.

Sanghera, M. K., et al. (1984). Electrophysiological properties of mouse dopamine neurons: In vivo and in vitro studies. *Neuroscience* **12**(3): 793-801.

Sarantis, M., et al. (1988). A presynaptic action of glutamate at the cone output synapse. *Nature* **332**(6163): 451-453.

Satake, W., et al. (2009). Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nature Genetics* **41**(12): 1303-1307.

Schikorski, T. and Stevens, C. F. (1997). Quantitative ultrastructural analysis of hippocampal excitatory synapses. *The Journal of Neuroscience* **17**(15): 5858-5867.

Schmitz, Y. (2003). Presynaptic regulation of dopaminergic neurotransmission. *Journal of Neurochemistry* **87**(2): 273.

Schmitz, Y., et al. (2002). Altered dopamine release and uptake kinetics in mice lacking D2 receptors. *The Journal of Neuroscience* **22**(18): 8002-8009.

Schoffelmeer, A. N., et al. (1986). Muscarine receptor-mediated modulation of [3H]dopamine and [14C]acetylcholine release from rat neostriatal slices: Selective antagonism by gallamine but not pirenzepine. *European Journal of Neuroscience* **128**(3): 291-294.

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

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

Schultz, W. (2007). Multiple dopamine functions at different time courses. *Annual review of Neuroscience*. **30:** 259-288.

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

Schultz, W., et al. (1997). A neural substrate of prediction and reward. *Science* **275**(5306): 1593-1599.

Schweizer, F. E. and Ryan, T. A. (2006). The synaptic vesicle: Cycle of exocytosis and endocytosis. *Current Opinion in Neurobiology* **16**(3): 298-304.

Senior, S. L., et al. (2008). Increased striatal dopamine release and hyperdopaminergic-like behaviour in mice lacking both alpha-synuclein and gamma-synuclein. *European Journal of Neuroscience* **27**(4): 947-957.

Seutin, V., et al. (1993). Apamin increases NMDA-induced burst-firing of rat mesencephalic dopamine neurons. *Brain Research* **630**(1–2): 341-344.

Sharkey, J., et al. (1988). Cocaine binding at σ receptors. *European Journal of Pharmacology* **149**(1–2): 171-174.

Shen, W., et al. (2008). Dichotomous dopaminergic control of striatal synaptic plasticity. *Science* **321**(5890): 848-851.

Shen, W., et al. (2005). Cholinergic suppression of KCNQ channel currents enhances excitability of striatal medium spiny neurons. *The Journal of Neuroscience* **25**(32): 7449-7458.

Shen, W., et al. (2007). Cholinergic modulation of Kir2 channels selectively elevates dendritic excitability in striatopallidal neurons. *Nature Neuroscience* **10**(11): 1458-1466.

Shepard, P. D. and Stump, D. (1999). Nifedipine blocks apamin-induced bursting activity in nigral dopamine-containing neurons. *Brain Research* **817**(1–2): 104-109.

Shulman, J. M., et al. (2011). Parkinson's disease: Genetics and pathogenesis. *Annual Review of Pathology: Mechanisms of Disease* **6**(1): 193-222.

Sidhu, A., et al. (2004). α-synuclein regulation of the dopaminergic transporter: A possible role in the pathogenesis of Parkinson's disease. *FEBS Letters* **565**(1–3): 1-5.

Sigrist, S. J. and Schmitz, D. (2011). Structural and functional plasticity of the cytoplasmic active zone. *Current Opinion in Neurobiology* **21**(1): 144-150.

Simon-Sanchez, J., et al. (2009). Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nature Genetics* **41**(12): 1308-1312.

Simon, N. W., et al. (2007). Cocaine exposure causes long-term increases in impulsive choice. *Behavioural Neuroscience* **121**(3): 543-549.

Singleton, A. B., et al. (2003). α-synuclein locus triplication causes Parkinson's disease. *Science* **302**(5646): 841.

Smith, Y., et al. (1998). Microcircuitry of the direct and indirect pathways of the basal ganglia. *Neuroscience* **86**(2): 353-387.

Soleng, A. F., et al. (2004). Activity-dependent excitability changes in hippocampal CA3 cell schaffer axons. *The Journal of Physiology* **560**(2): 491-503.

Somogyi, P., et al. (1981). Monosynaptic input from the nucleus accumbens-ventral striatum region to retrogradely labelled nigrostriatal neurones. *Brain Research* **217**(2): 245-263.

Sonders, M. S. and Amara, S. G. (1996). Channels in transporters. *Current Opinion in Neurobiology* **6**(3): 294-302.

Sonders, M. S., et al. (1997). Multiple ionic conductances of the human dopamine transporter: The actions of dopamine and psychostimulants. *The Journal of Neuroscience* **17**(3): 960-974.

Specht, C. and Schoepfer, R. (2001). Deletion of the alpha-synuclein locus in a subpopulation of C57Bl/6J inbred mice. *BMC Neuroscience* **2**(1): 11.

Spillantini, M. G., et al. (1997). [alpha]-synuclein in lewy bodies. *Nature* **388**(6645): 839-840.

Stacy, M. (2011). Nonmotor symptoms in Parkinson's disease. *International Journal of Neuroscience* **121**(S2): 9-17.

Staines, W. A., et al. (1981). Demonstration of a pallidostriatal pathway by retrograde transport of HRP-labeled lectin. *Brain Research* **206**(2): 446-450.

Stamford, J., et al. (1995). Fast cyclic voltammetry in brain slices. *Neuromethods: Voltammetric methods in brain systems*. Boulton,A, Baker, G. and Adams, R. Humana.

Stamford, J. A., et al. (1989). Dissociation of the actions of uptake blockers upon dopamine overflow and uptake in the rat nucleus accumbens: *In vivo* voltammetric data. *Neuropharmacology* **28**(12): 1383-1388.

Steffensen, S. C., et al. (2008). Cocaine disinhibits dopamine neurons in the ventral tegmental area via use-dependent blockade of GABA neuron voltage-sensitive sodium channels. *European Journal of Neuroscience* **28**(10): 2028-2040.

Stevens, C. F. and Tsujimoto, T. (1995). Estimates for the pool size of releasable quanta at a single central synapse and for the time required to refill the pool. *Proceedings of the National Academy of Sciences* **92**(3): 846-849.

Stuber, G. D., et al. (2008). Reward-predictive cues enhance excitatory synaptic strength onto midbrain dopamine neurons. *Science* **321**(5896): 1690-1692.

Südhof, T. C. (2004). The synaptic vesicle cycle. *Annual Review of Neuroscience* **27**: 509-547.

Südhof, T. C. and Rizo, J. (2011). Synaptic vesicle exocytosis. *Cold Spring Harbor Perspectives in Biology* **3**(12).

Südhof, T. C. and Rothman, J. E. (2009). Membrane fusion: Grappling with snare and SM proteins. *Science* **323**(5913): 474-477.

Sugita, S., et al. (1991). Distinct muscarinic receptors inhibit release of gamma-aminobutyric acid and excitatory amino acids in mammalian brain. *Proceedings of the National Academy of Sciences* **88**(6): 2608-2611.

Sullivan, M. A., et al. (2008). Recurrent inhibitory network among striatal cholinergic interneurons. *The Journal of Neuroscience* **28**(35): 8682-8690.

Surmeier, D. J. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends in Neurosciences* **30**(5): 228.

Surmeier, D. J., et al. (1995). Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* **14**(2): 385-397.

Surmeier, D. J., et al. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends in Neurosciences* **30**(5): 228-235.

Surmeier, D. J., et al. (1992). Dopamine receptor subtypes colocalize in rat striatonigral neurons. *Proceedings of the National Academy of Sciences* **89**(21): 10178-10182.

Surmeier, D. J., et al. (1996). Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. *The Journal of Neuroscience.* **16**(20): 6579-6591.

Suzuki, T., et al. (2001). Dopamine-dependent synaptic plasticity in the striatal cholinergic interneurons. *The Journal of Neuroscience* **21**(17): 6492-6501.

Tang, J., et al. (2006). A complexin/synaptotagmin 1 switch controls fast synaptic vesicle exocytosis. *Cell* **126**(6): 1175-1187.

Tepper, J. M., et al. (2004). GABAergic microcircuits in the neostriatum. *Trends in Neurosciences* **27**(11): 662-669.

Tepper, J. M., et al. (2010). Heterogeneity and diversity of striatal GABAergic interneurons. *Frontiers in Neuroanatomy* **4**.150

Testa, C., et al. (1994). Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. *The Journal of Neuroscience* **14**(5): 3005-3018.

Thomas, M. J., et al. (2001). Long-term depression in the nucleus accumbens: A neural correlate of behavioral sensitization to cocaine. *Nature Neuroscience* **4**(12): 1217-1223.

Thomas, T. M., et al. (2000). Cortical inputs to M2-immunoreactive striatal interneurons in rat and monkey. *Synapse* **37**(4): 252-261.

Thomson, A. M. (1997). Activity-dependent properties of synaptic transmission at two classes of connections made by rat neocortical pyramidal axons in vitro. *The Journal of Physiology* **502**(Pt 1): 131-147.

Thomson, A. M. (2000). Molecular frequency filters at central synapses. *Progress in Neurobiology* **62**(2): 159-196.

Thomson, A. M. and Bannister, A. P. (1999). Release-independent depression at pyramidal inputs onto specific cell targets: Dual recordings in slices of rat cortex. *The Journal of Physiology* **519**(1): 57-70.

Thomson, A. M., et al. (1993). Large, deep layer pyramid-pyramid single axon epsps in slices of rat motor cortex display paired pulse and frequency-dependent depression, mediated presynaptically and self-facilitation, mediated postsynaptically. *Journal of Neurophysiology* **70**(6): 2354-2369.

Thomson, A. M., et al. (1993). Single axon excitatory postsynaptic potentials in neocortical interneurons exhibit pronounced paired pulse facilitation. *Neuroscience* **54**(2): 347-360.

Threlfell, S., et al. (2010). Striatal muscarinic receptors promote activity dependence of dopamine transmission via distinct receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. *The Journal of Neuroscience* **30**(9): 3398-3408.

Threlfell, S., et al. (2012). Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. *Neuron* **75**(1): 58-64.

Tobler, P. N., et al. (2005). Adaptive coding of reward value by dopamine neurons. *Science* **307**(5715): 1642-1645.

Torres, G. E. (2006). The dopamine transporter proteome. *Journal of Neurochemistry* **97**: 3-10.

Torres, G. E., et al. (2003). Plasma membrane monoamine transporters: Structure, regulation and function. *Nature Reviews Neuroscience* **4**(1): 13-25.

Tozzi, A., et al. (2011). Mechanisms underlying altered striatal synaptic plasticity in old A53T-α synuclein overexpressing mice. *Neurobiology of Aging* **33** (8).1792-1799

Tsai, H.-C., et al. (2009). Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. *Science* **324**(5930): 1080-1084.

Ungless, M. A., et al. (2004). Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science* **303**(5666): 2040-2042.

Ungless, M. A., et al. (2001). Single cocaine exposure *in vivo* induces long-term potentiation in dopamine neurons. *Nature* **411**(6837): 583-587.

Usiello, A., et al. (2000). Distinct functions of the two isoforms of dopamine D2 receptors. *Nature* **408**(6809): 199-203.

Van Petegem, F. and D. L. J. Minor (2006). The structural biology of voltage-gated calcium channel function and regulation. *Biochemical Society Transactions* **34**(5): 887-893.

Venton, B. J., et al. (2006). Cocaine increases dopamine release by mobilization of α synapsin-dependent reserve pool. *The Journal of Neuroscience* **26**(12): 3206-3209.

Venton, B. J., et al. (2003). Real-time decoding of dopamine concentration changes in the caudate–putamen during tonic and phasic firing. *Journal of Neurochemistry* **87**(5): 1284-1295.

Vilchis, C., et al. (1999). Ca2+ channels that activate Ca2+-dependent K+ currents in neostriatal neurons. *Neuroscience* **95**(3): 745-752.

Wadiche, J. I., et al. (1995). Ion fluxes associated with excitatory amino acid transport. *Neuron* **15**(3): 721-728.

Waelti, P., et al. (2001). Dopamine responses comply with basic assumptions of formal learning theory. *Nature* **412**(6842): 43-48.

Wanat, M. J., et al. (2009). Phasic dopamine release in appetitive behaviors and drug abuse. *Current Drug Abuse Reviews* **2**(2): 195-213.

Wang, H. and Pickel, V. M. (2002). Dopamine D2 receptors are present in prefrontal cortical afferents and their targets in patches of the rat caudate-putamen nucleus. *The Journal of Comparative Neurology* **442**(4): 392-404.

Wang, Z., et al. (2006). Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. *Neuron* **50**(3): 443-452.

Watabe-Uchida, M., et al. (2012). Whole-brain mapping of direct inputs to midbrain dopamine neurons. *Neuron* **74**(5): 858-873.

Weiner, D. M., et al. (1990). Expression of muscarinic acetylcholine and dopamine receptor mrnas in rat basal ganglia. *Proceedings of the National Academy of Sciences* **87**(18): 7050-7054.

Wersinger, C. and Sidhu, A. (2003). Attenuation of dopamine transporter activity by α-synuclein. *Neuroscience Letters* **340**(3): 189-192.

Wersinger, E., et al. (2006). The glutamate transporter EAAT5 works as a presynaptic receptor in mouse rod bipolar cells. *The Journal of Physiology* **577**(1): 221-234.

Wieczorek, W. J. and Kruk, Z. L. (1994). A quantitative comparison on the effects of benztropine, cocaine and nomifensine on electrically evoked dopamine overflow and rate of re-uptake in the caudate putamen and nucleus accumbens in the rat brain slice. *Brain Research* **657**(1–2): 42-50.

Wightman, R. M., et al. (1988). Real-time characterization of dopamine overflow and uptake in the rat striatum. *Neuroscience* **25**(2): 513-523.

Wightman, R. M. and Zimmerman, J. B. (1990). Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake. *Brain Research* **15**(2): 135-144.

Wilson, C., et al. (1990). Firing patterns and synaptic potentials of identified giant aspiny interneurons in the rat neostriatum. *The Journal of Neuroscience* **10**(2): 508-519.

Wilson, C. J. and Goldberg , J. A. (2006). Origin of the slow afterhyperpolarization and slow rhythmic bursting in striatal cholinergic interneurons. *Journal of Neurophysiology* **95**(1): 196-204.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience* **5**(6): 483-494.

Wise, R. A. and Schwartz, H. V. (1981). Pimozide attenuates acquisition of lever-pressing for food in rats. *Pharmcology, Biochemistry and Behaviour* **15**(4): 655-656.

Wise, R. A., et al. (1978). Neuroleptic-induced "anhedonia" in rats: Pimozide blocks reward quality of food. *Science* **201**(4352): 262-264.

Witten, I. B., et al. (2010). Cholinergic interneurons control local circuit activity and cocaine conditioning. *Science* **330**(6011): 1677-1681.

Wolf, M. E. and Roth, R. H. (1990). Autoreceptor regulation of dopamine synthesis. *Annals of the New York Academy of Sciences* **604**(1): 323-343.

Wolfart, J., et al. (2001). Differential expression of the small-conductance, calcium-activated potassium channel SK3 is critical for pacemaker control in dopaminergic midbrain neurons. *J. Neurosci.* **21**(10): 3443-3456.

Wonnacott, S. (1997). Presynaptic nicotinic ACh receptors. *Trends in Neurosciences* **20**(2): 92-98.

Woodward, J. J., et al. (1995). In vitro and in vivo effects of cocaine and selected local anesthetics on the dopamine transporter. *European Journal of Pharmacology* **277**(1): 7-13.

Wreggett, K. A. and Seeman, P. (1984). Agonist high- and low-affinity states of the D2-dopamine receptor in calf brain. Partial conversion by guanine nucleotide. *Molecular Pharmacology* **25**(1): 10-17.

Wu, Q., et al. (2001). Preferential increases in nucleus accumbens dopamine after systemic cocaine administration are caused by unique characteristics of dopamine neurotransmission. *The Journal of Neuroscience* **21**(16): 6338-6347.

Wu, Y., et al. (2000). The organization of the striatal output system: A single-cell juxtacellular labeling study in the rat. *Neuroscience Research* **38**(1): 49-62.

Xu, J. and Wu, L.-G. (2005). The decrease in the presynaptic calcium current is a major cause of short-term depression at a calyx-type synapse. *Neuron* **46**(4): 633-645.

Yan, Z., et al. (2001). Coordinated expression of muscarinic receptor messenger RNAs in striatal medium spiny neurons. *Neuroscience* **103**(4): 1017-1024.

Yan, Z., et al. (1999). Protein phosphatase 1 modulation of neostriatal AMPA channels: Regulation by DARPP-32 and spinophilin. *Nature Neuroscience* **2**(1): 13-17.

Yan, Z., et al. (1997). D2 dopamine receptors reduce N-type Ca2+ currents in rat neostriatal cholinergic interneurons through a membrane-delimited, protein-kinase-C-insensitive pathway. *Journal of Neurophysiology* **77**(2): 1003-1015.

Yavich, L., et al. (2005). Locomotor activity and evoked dopamine release are reduced in mice overexpressing A30P-mutated human [alpha]-synuclein. *Neurobiology of Disease* **20**(2): 303-313.

Yavich, L., et al. (2004). Role of {alpha}-synuclein in presynaptic dopamine recruitment. *The Journal of Neuroscience* **24**(49): 11165-11170.

Yin, H. H. (2006). The role of the basal ganglia in habit formation. *Nature Reviews. Neuroscience* **7**(6): 464.

Yin, H. H. and Knowlton, B. J. (2006). The role of the basal ganglia in habit formation. *Nature Reviews Neuroscience* **7**(6): 464-476.

Yizhar, O., et al. (2011). Optogenetics in neural systems. *Neuron* **71**(1): 9-34.

Yung, K. K. L., et al. (1995). Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: Light and electron microscopy. *Neuroscience* **65**(3): 709-730.

Zarranz, J. J., et al. (2004). The new mutation, E46K, of α-synuclein causes parkinson and lewy body dementia. *Annals of Neurology* **55**(2): 164-173.

Zhang, H. and Sulzer, D. (2003). Glutamate spillover in the striatum depresses dopaminergic transmission by activating group I metabotropic glutamate receptors. *The Journal of Neuroscience* **23**(33): 10585-10592.

Zhang, H. and Sulzer, D. (2004). Frequency-dependent modulation of dopamine release by nicotine. *Nature Neuroscience* **7**(6): 581.

Zhang, L., et al. (2009). Controls of tonic and phasic dopamine transmission in the dorsal and ventral striatum. *Molecular Pharmacology* **76**(2): 396-404.

Zhang, W., et al. (2002). Multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release, as studied with M1–M5 muscarinic receptor knock-out mice. *The Journal of Neuroscience* **22**(15): 6347-6352.

Zhao, S., et al. (2011). Cell type–specific channelrhodopsin-2 transgenic mice for optogenetic dissection of neural circuitry function. *Nature Methods* **8**(9): 745-752.

Zheng, T. and Wilson, C. J. (2002). Corticostriatal combinatorics: The implications of corticostriatal axonal arborizations. *Journal of Neurophysiology* **87**(2): 1007-1017.

Zhou, F., et al. (2001). Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nature Neuroscience* **4**: 1224.

Zoli, M., et al. (1998). The emergence of the volume transmission concept. *Brain Research. Brain Research Reviews* **26**(2-3): 136-147.

Zucker, R. S. and Regehr, W. G. (2002). Short-term synaptic plasticity. *Annual Review of Physiology* **64**(1): 355-405.