Presynaptic control of corticostriatal inputs: role of GABA

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List of abbreviations

ACh: acetylcholine

AHP: afterhyperpolarisation

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ATP: adenosine tri-phosphate

AP: action potential

BG: basal ganglia

Bz: benzodiazepine

CAMKIIa: calmodulin-dependent protein kinase type II

cGMP: cyclic guanosine monophosphate

CRI: calretinin-positive interneuron

DAT: dopamine transporter

GABA: γ-aminobutyric acid

GFP: green fluorescent protein

GP(e/i): external/internal globus pallidus

EPN: entopeduncular nucleus

FSI: fast spiking interneuron

GAD: glutamic acid decarboxylase

GPCR: G-protein coupled receptor

LAI: large aspiny interneuron

LTSI: low threshold-spiking interneuron

m/nAChR: muscarinic/nicotinic acetylcholine receptor

MSN: medium spiny neuron

NAc: nucleus accumbens

NMDA: N-methyl-D-aspartate

m/nAChR: muscarinic/nicotinic acetylcholine receptor

NGFI: neurogliaform interneuron

NO: nitric oxide

nNOS: neuronal nitric oxide synthase

NSF: N-ethylmaleimide-sensitive ATPase

NPY: neuropeptide Y

ODQ: H-[1,2,4]oxadiazolo[4, 3-a]quinoxalin-1-one

PP/D/F/R: paired pulse depression/facilitation/ratio

sGC: soluble guanylate cyclase

SNAP: synaptosomal associated protein

SN(c/r): substantia nigra (pars compacta/pars reticulata)

SSN: somatostatin

STN: subthalamic nucleus

TAN; tonically active neuron

THI: tyrosine hydroxylase-positive interneuron

VGluT1/2: vesicular glutamate transporter 1/2

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Abstract

The basal ganglia (BG) are a group of nuclei in the basal forebrain critical in movement, goal-directed behaviour and action selection. Cortical projections to the largest BG nucleus, the striatum, are highly important in theories of BG function. Therefore, we have investigated the role of striatal neurons in modulating the activity of corticostratal synapses. In an *in-vitro* preparation of rodent brain slices, we conducted whole-cell patch clamp recordings of single and pairs of striatal neurons and recorded responses of medium spiny neurons (MSNs) to stimulation of corticostriatal fibres. In the presence of opioid, $GABA_A$, NK1 and cholinergic receptor antagonists, antidromic stimulation of a population of MSNs (5 stims, 50 Hz) caused suppression of subsequently evoked EPSPs in MSNs. This suppression was dependent upon the interval between antidromic MSN stimulation and the stimulation of evoked EPSPs; suppression was larger at 500 ms intervals than at 1 or 2 s intervals. These effects were completely blocked by the $GABA_B$ antagonist CGP 52432. Bursts of evoked action potentials (5 APs, 50 Hz) in a single MSN were insufficient to cause these effects in a nearby MSN. Similar spikes in single fast spiking interneurons and low threshold spiking interneurons (LTSIs) were also insufficient. Conversely, single neurogliaform interneurons (NGFIs) could suppress evoked EPSPs in nearby MSNs in a $GABA_B$-dependent manner. This suppression was more likely in NGFI-MSN pairs that exhibited direct GABAergic interactions. We also tested long depolarisations in LTSIs, a protocol that preferentially releases NO, which was shown to suppress evoked EPSPs through a non-GABAergic mechanism. Finally, we tested the application of exogenous NPY to slices, which also inhibited corticostriatal transmission. These results provide the first demonstration of how $GABA_B$ receptors at corticostriatal synapses are activated by endogenous GABA released by striatal neurons. They also reveal novel mechanisms through which striatal factors influence these synapses.

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Chapter One: Introduction

The basal ganglia are a group of nuclei in the basal forebrain responsible for a variety of essential basic functions. The dorsal structures are responsible for action selection, goal directed motor control and associated learning while the ventral structures are involved in limbic function (Bolam *et al.*, 2000; Tepper & Bolam, 2004; McHaffie *et al.*, 2005). The structure and function of the basal ganglia is phylogenetically well conserved across mammalian and some non-mammalian species (Medina & Reiner, 1995; Luo *et al.*, 2001; Grillner *et al.*, 2013), indicating an ancient and crucial role in motor control. Indeed, these nuclei are the primary area affected in motor diseases such as Parkinson's and Huntington's disease, where the most fundamental movements are seriously impaired. For this reason, the underlying function of the basal ganglia has come under increasing scrutiny from researchers.

The following introduction presents a background on the basal ganglia in general and the striatum in particular, with emphasis on striatal neurons and projections to the striatum from the cortex.

1.1 Basal ganglia organization and function

The basal ganglia (BG) are composed of four major nuclei: The striatum, globus pallidus (GP), substantia nigra (SN) and subthalamic nucleus (STN). Between primates and rodents, basal ganglia structures are anatomically distinct yet their respective functions remain conserved (Parent & Hazrati, 1995).

In rodents, the BG are comprised of: the neostriatum (including dorsal and ventral regions), the GP and the entopeduncular nucleus (EPN). The SN is divided into two regions, the pars compacta (SNc) and the pars reticulate (SNr). In primates, the GP is divided into internal and external segments, termed GPi and GPe respectively. The primate GPe is functionally similar to the rodent EPN and the primate GPi is functionally similar to the rodent GP.

As both the recipient of BG inputs and as the body with most diverse neuron population, the striatum is the largest and most important of the basal ganglia nuclei. Thus it is regarded as the primary centre for integration and processing of information and facilitation of movement. The striatum receives excitatory input

primarily from the cortex and thalamus; these account for around 85% of all striatal synapses, with 90% of corticostriatal synapses terminating onto striatum principal cells, the medium spiny neuron (MSN) (Wilson, 2004). The remainder terminate onto striatal interneurons.

Striatal information is transmitted to the BG output nuclei, which are the SNr/GPi in primates and SNr/EPN in rodents (detailed below). This occurs through two major pathways: the *direct* and *indirect* pathways (Smith *et al.*, 1998). In the direct pathway, stiratonigral MSNs provide direct monosynaptic inhibition of the principal cells of the output nuclei. In contrast, the indirect pathway is comprised of a more complex pathway whereas striatopallidal MSNs project to the GP which in turn project to EPN/SNr directly and also via the STN (Smith *et al.*, 1998). Since these output nuclei themselves tonically suppress movement, the overall result of excitation of the direct pathway is to facilitate movement whereas excitation of the indirect pathway suppresses movement. The MSNs that give rise to the two pathways are functionally similar and thus are generally considered as a single class of neuron, however differences exist that allow discrete subtypes to emerge. For example, striatonigral MSNs express almost exclusively D1 dopamine receptors and release substance P and dynorphin, whereas striatopallidal MSNs express almost exclusively D2 dopamine receptors and release enkephalin (Gerfen *et al.*, 1990; Bolam *et al.*, 2000). Differences between subtypes are explained further in section 1.9.1. These dopamine receptors respond to tonic dopaminergic input from afferent SNc fibres and ventral tegmental area (VTA) fibres (Haber *et al.*, 2000).

The other entry point for information into the basal ganglia is cortical and thalamic input into the STN. Cortical inputs originate from motor regions with thalamic input originating from intralaminar nuclei (Kitai & Deniau, 1981; Afsharpour, 1985; Bevan *et al.*, 1995). Due to the glutamatergic STN projections to the output nuclei, this connection is the fastest through which the cortex and thalamus can influence basal ganglia output (Kita, 1994) and is sometimes referred to as the *hyperdirect pathway*.

The output nuclei of the basal ganglia, the substantia nigra pars reticulata (SNr) and GPi/EPN, are homogenous structures with neurons that are tonically active (Nakanishi *et al.*, 1987). These neurons project primarily to the ventral thalamus (Ilinsky *et al.*, 1997) but also to parts of the brainstem (Tepper *et al.*, 2007). SNr

and GPi/EPN neurons are GABAergic, therefore effecting a tonic suppression of targeted motor pathways (Chevalier & Deniau, 1982). These output nuclei receive inputs from striatal pathways that suppress the tonic firing of neurons, thus disinhibiting motor pathways (Deniau & Chevalier, 1985). In addition, the SNr also projects local afferents to the SNc, regulating the nigrostriatal pathway (Tepper *et al.*, 2002).

This series of inputs and outputs through the basal ganglia has resulted in the *action selection hypothesis*. Salient sensory information, such as those that result from a sudden noise or an important visual event, result in excitatory input into the striatum from both cortical and subcortical sources (particularly via the thalamus). These inputs and resulting outputs are the basis of collection of parallel looped architectures that include cortex, thalamus and many hindbrain and midbrain structures that compete for motor control (Alexander *et al.*, 1986; McHaffie *et al.*, 2005). The hub of these loops is the striatum, and its intrinsic architecture and organisation allow it to select from competing attempts for motor control (Alexander *et al.*, 1986; McHaffie *et al.*, 2005). The action selection hypothesis is detailed further in section 1.8.

1.2 Striatum

So called due to the appearance of dense striato-pallidal axon bundles that traverse the structure, the striatum is the largest and most complex of the basal ganglia nuclei. The striatum is divided into its dorsal and ventral regions. In primates the dorsal region is divided by the internal capsule into lateral putamen and medial caudate nucleus. In rodents, no clear distinction exists between dorsolateral and dorsomedial striatum, sometimes being referred to as caudate-putamen (Paxinos & Wilson, 1998). The ventral striatum is comprised of NAc, striatal cells of the olfactory tubercle and anterior perforated substance.

The striatum is populated by a dense network of neuron types, most of which express GABA as the primary neurotransmitter. The primary neurons are the MSNs, whose main projections target the output nuclei but which also form intrastriatal collaterals with other MSNs. MSNs also release the co-transmitters substance P, enkephalin and dynorphin (Aronin *et al.*, 1981; Pickel *et al.*, 1982;

Bolam *et al.*, 1983; Bolam & Izzo, 1988; Pickel *et al.*, 1992). MSNs are covered in more detail in section 1.9.1.

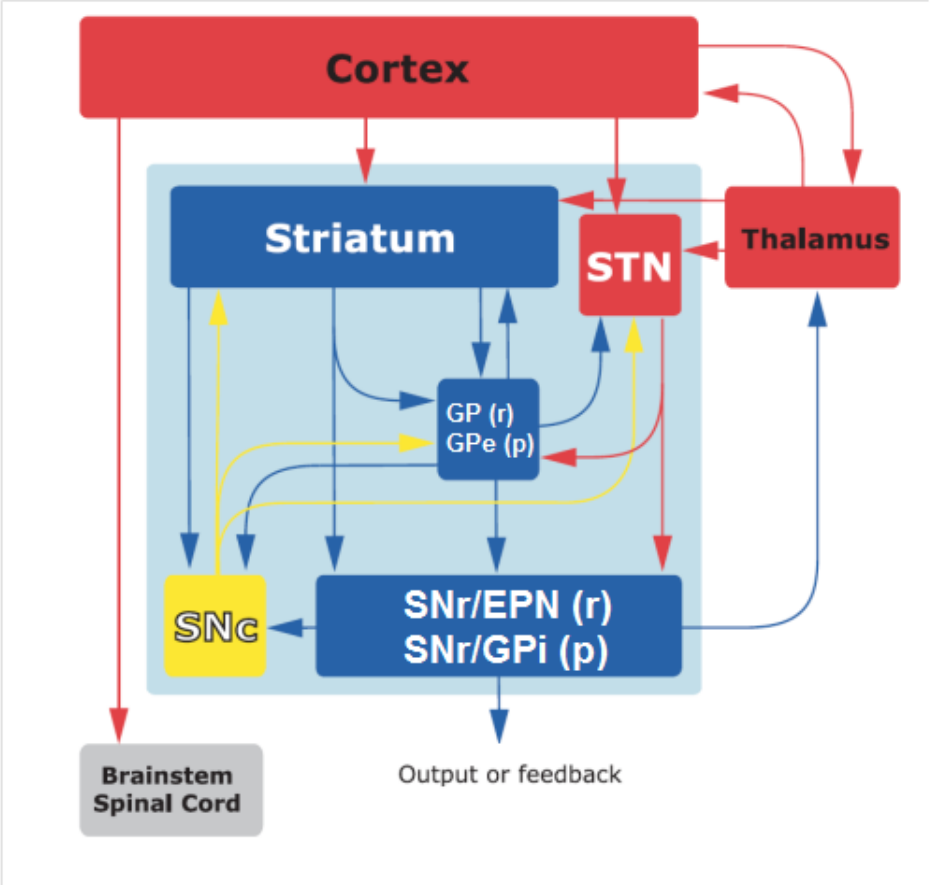

Figure 1.1: Neuron projections of the dorsal basal ganglia. The light blue box contains basal ganglia structures. The striatum receives most of the input into the basal ganglia and is the largest and most complex structure in the BG. The main inputs into the striatum arise from glutamatergic corticothalamic input to the striatum. Cortical inputs arise from across the cortex with both ipsilateral and contralateral connections, with thalamic inputs primarily from the intralaminar nuclei. The SNc provides lesser inputs into the striatum. The striatum itself projects to the output nulclei of the basal ganglia, the SNr and GPi in primates (p) and the SNr and EPN in rodents (r). These structures mediate basal ganglia effects via projections to subcortical "premotor" regions: the lateral habenula, pendunculopontine nucleus, reticular formation and superior colliculus. The output nuclei also project back to the ventral thalamus and thus indirectly feedback to the cortex. Red arrows; glutamatergic projections. Blue arrows; GABAergic projections. Yellow arrows; dopaminergic projections. EPN: entopeduncular nucleus; SN(c/r); substantia nigra (pars compacta/pars reticulata) STN; subthalamic nucleus. Adapted from Tepper *et al.* (2007)

In contrast to MSNs that project to other brain regions, the striatum also exhibits several neuron types that project only within the striatum, termed interneurons. Classically, three main interneuron types were evident: fast-spiking interneurons (FSIs) and low-threshold spiking interneurons (LTSIs) are GABAergic; large aspiny interneurons (LAIs) express acetylcholine (Kawaguchi, 1993). Recently, however, other interneuron types have become apparent. Neurogliaform interneurons (NGFIs) and tyrosine hydroxylase-positive interneurons (THIs) release GABA; calretinin-positive interneurons are less well characterised (Chesselet & Graybiel, 1986; Bennett & Bolam, 1993; Ibanez-Sandoval *et al.*, 2010, 2011). These neurons are discussed in more detail in section 1.9.

The striatum exhibits several layers of functional organisation related to its neurochemistry and anatomical connections (Gerfen, 1992). First, the major axon projections of striatal MSNs form two major pathways: the *direct pathway* and *indirect pathway*. Direct pathway MSNs form inhibitory connections with the output nuclei. Indirect pathway MSNs form inhibitory connections with the GPe, with these GPe neurons forming inhibitory connections with the output nuclei (Alexander & Crutcher, 1990; DeLong, 1990; Smith *et al.*, 1998). Since the output nuclei are tonically active (DeLong, 1972; DeLong *et al.*, 1985; Nakanishi *et al.*, 1987), firing of direct pathway MSNs suppresses output nuclei activity and facilitates movement, whilst firing of indirect pathway MSNs disinhibits output nuclei and reduces movement (Albin *et al.*, 1989; Alexander & Crutcher, 1990; DeLong, 1990; Kravitz *et al.*, 2010).

Second, based upon neurochemical markers, the striatum has been shown to exhibit a structural dichotomy known as patch-matrix compartmentalization. Striatal patches, also referred to as *striosomes*, are discrete regions within the striatum that represent ~11% of striatal volume (Graybiel & Ragsdale, 1979; Zheng & Wilson, 2002). Several markers are used for striosome staining, including reduced acetylcholinesterase activity, elevated μ-opioid receptor binding, increased SP and enkephalin expression, reduced expression of Ca^{2+} binding protein and a lower proportion of LTSIs (Graybiel & Ragsdale, 1978; Herkenham & Pert, 1981; Gerfen *et al.*, 1985). A greater proportion of MSNs from these regions project to the substantial nigra, with MSNs projecting to the SNc rather than SNr (Gerfen, 1984; Gerfen *et al.*, 1985; Bolam *et al.*, 1988). Generally, matrix exhibits the opposite features and comprises the remaining 89%, however recent data have shown that regions delineated by striosomal markers often do not overlap. For example, one report (Tajima & Fukuda, 2013) has shown that two markers for patch, SP and μ-

opioid receptor, do not produce a uniform overlap when both are tested simultaneously in striatal tissue.

Third, the striatum receives massive glutamatergic input from all over the cortical mantle in a topographical fashion (Parent & Hazrati, 1995) and from several thalamic nuclei (McFarland & Haber, 2000). Major dopaminergic input to the striatum arises from the SNc and VTA (Haber *et al.*, 2000), providing a tonic input of dopamine.

1.3 Output nuclei

Direct pathway striatal neuron projections terminate upon neurons of the substantia nigra pars reticulata (SNr) and the internal globus pallidus (GPi), otherwise known as the entopenduncular nucleus (EPN) in rodents. These areas are the last stage for information processing before leaving the BG network and thus are referred to as the output nuclei. The neurons in these areas are the main targets for MSNs of the direct pathway and since there are many more direct pathway MSNs than output neurons, a high degree of convergence is likely (Smith *et al.*, 1998). These synapses comprise 70% of all afferent GPi/EPN synapses (Kim *et al.*, 1976). The SNr and GPi/EPN also receive input from GPe neurons, which are also GABAergic (Smith *et al.*, 1998; Deniau *et al.*, 2007), thus allowing SNr neurons to be disinhibited by indirect pathway MSNs. SNr neurons also receive glutamatergic input from STN and cortical trans-striatal pathways (Kita & Kitai, 1987; Kolomiets *et al.*, 2003)

Both brain structures contain neurons that are tonically active both *in vivo* and *in vitro*, exerting continuous GABAergic inhibition of neurons they innervate (Oertel *et al.*, 1984; DeLong *et al.*, 1985; Nakanishi *et al.*, 1987, 1990, 1991; Ilinsky *et al.*, 1997; Kita, 2001; Surmeier *et al.*, 2005; Hikosaka, 2007). GPi/EPN neurons project to the ventral anterior/ventral thalamic complex and to the centromedian (CM) in primates and onto GABAergic interneurons (Kuo & Carpenter, 1973; Kim *et al.*, 1976; Ilinsky *et al.*, 1997). SNr neurons inhibit thalamic ventromedial and parafascicular nuclei and also suppress the superior colliculus (SC) and the pendunculopontine nucleus (Gerfen *et al.*, 1982; Nakanishi *et al.*, 1987; Deniau & Chevalier, 1992). In particular, the SNr-SC connection has been studied in order to

better understand BG output systems as the neuronal mechanisms of the SC are already well understood and this efferent connection is relatively less complex compared to other SNr targets. SC neurons are critical in oculomotor function, with SNr neuron cessation having been shown to be necessary for preparation of saccadic eye movements. Conversely, blocking of $GABA_A$ receptors in SNr neurons renders primates unable to suppress voluntary eye saccades (Hikosaka & Wurtz, 1985a, 1985b; Hauber, 1998). These results demonstrate that SNr neurons act to gate the continuous excitatory input to the SC from other brain areas (Hikosaka *et al.*, 2000; Hikosaka, 2007). In this manner the physiological activity of the SNr-SC connection is a simplification of the activity of the output nuclei generally.

1.4 Other basal ganglia nuclei

1.4.1 External globus pallidus

The external globus pallidus (GPe), known simply as the globus pallidus (GP) in rodents, is an important BG structure due to its role in the indirect pathway. Located medial and caudal to the striatum, neurons of the GP are GABAergic (Smith *et al.*, 1987; Kita, 1994; Kita & Kitai, 1994). The two primary sources of input to the GPe are: the striatal indirect pathway and the STN. The GPe is the main target of GABAergic indirect pathway neurons but also receives collaterals from direct pathway MSNs (Kawaguchi *et al.*, 1990; Smith *et al.*, 1998; Fujiyama *et al.*, 2011), however the functional connectivity of direct pathway collaterals has been called into question (Chuhma *et al.*, 2011). The other major afferent arises from STN neurons and is glutamatergic (Shink & Smith, 1995). Much sparser inputs from cerebral cortex, intralaminar thalamic nuclei and SNc exists (Hazrati *et al.*, 1990; Kita & Kitai, 1994; Parent & Hazrati, 1995; Yasukawa *et al.*, 2004). 80% of synapses on GPe neuron dendrites are symmetric synapses, presumed GABAergic. Of these, 80% are of striatal origin and 20% formed from local axon collaterals. The remaining 20% dendritic synapses are asymmetric and primarily arise from STN fibres (Kita & Kitai, 1994; Shink & Smith, 1995; Kita, 2007).

At least two neuron types are expressed in the GPe: parvalbumin-positive and parvalbumin-negative neurons. Parvalbumin-negative neurons comprise 33% of GPe neurons and project mainly to the striatum. These pallidostriatal synapses specifically target paravalbumin-positive and NOS-positive interneurons (Bevan *et al.*, 1998).

Their dendrites are sparsely dotted with spines. Parvalbumin-positive neurons do not project to striatum and express aspiny dendrites (Kita & Kitai, 1994; Kita & Kita, 2001; Kita, 2007). Both subtypes project to STN and GPi/EPN (Kita & Kitai, 1994; Bevan *et al.*, 1998) with a small number also innervating lesser connections to the dorsal thalamus, inferior colliculus and PPN (Bevan *et al.*, 1998; Kita, 2007; Sadek *et al.*, 2007).

1.4.2 Substantia nigra pars compacta

The substantia nigra pars compacta (SNc) is a structure in the midbrain that provides the major dopaminergic input to the striatum, forming the nigrostriatal pathway (Graybiel & Ragsdale, 1979; Aosaki *et al.*, 1994). This tonic input is critical in normal BG function as manifested by its absence as the primary cause of Parkinson's disease.

SNc neurons are spontaneously active with a firing rate of around 5 Hz *in vivo* (Yung *et al.*, 1991; Grillner & Mercuri, 2002; Brown *et al.*, 2009). They can be subdivided into dorsal and ventral neurons. Dorsal neurons are immunopositive for calbindin and have higher firing rates; ventral neurons are immunonegative for calbindin and fire more slowly (Smith & Kieval, 2000; Brown *et al.*, 2009). They receive GABAergic input from striatal patch direct pathway MSNs, GPe and SNr neurons (Bolam & Smith, 1990; Smith & Bolam, 1990b; Mailly *et al.*, 2003; Fujiyama *et al.*, 2011).

SNc projections to the striatum are unusual both in terms of their size and their ubiquity. Individual SNc neuron arborisations cover an average of 2.7% of striatum, with each neuron innervating both matrix and patch, although favouring one or the other (Matsuda *et al.*, 2009). Axon collaterals terminate uniformly across the

striatum, such that almost all striatal structures are innervated at both synaptic and extra-synaptic locations (Yung & Bolam, 2000; Moss & Bolam, 2008). Axons of the nigrostriatal pathway have been shown to form parallel synapses onto the dendritic shafts or the base of dendritic spines of MSNs (Tepper *et al.*, 2007), frequently converging with corticostriatal but not thalamostriatal terminals (Smith & Bolam, 1990a; Smith *et al.*, 1994). This dense axonal network permits the *volume transmission* of dopamine in the striatum. Volume transmission is the form of neurotransmission where, instead of the typical mechanism where neurotransmitters are released at discrete synapsis, neurotransmitter is released into the general extracellular space and activates many neurons at a general level. This method of dopamine release accounts for its omnipresent effects. Lesser projections to the STN and GPe also exist (Hassani *et al.*, 1997; Matsuda *et al.*, 2009).

Nigrostriatal connections have been studied particularly due to their relevance in Parkinson's disease pathogenesis and in reward. These projections have classically been known to release dopamine and modulate the activity of striatonigral and striatopalidal MSNs, which express D1 and D2 receptors respectively (Gerfen *et al.*, 1990; Surmeier *et al.*, 1996).

Dopamine neurons fire distinctive patters in response to reward-related events, particulary where unexpected reward is given or an expected reward is absent (Schultz, 1998; Bromberg-Martin *et al.*, 2010). Whilst the exact role of dopamine in these processes remains elusive, dopamine has been implicated in cholinergic interneuron burst-pause responses (Goldberg & Reynolds, 2011). An increase in dopamine release from SNc neurons is coincident with LAI pauses (Morris *et al.*, 2004) and when the nigrostiratal pathway is lesioned, LAI burst-pause responses are absent (Aosaki *et al.*, 1994).

Thus the role of dopamine in the nigrostriatal pathway is both vital and complex. Not only is it implicated in reward pathways, it mediates complex effects upon individual neurons, improves signal-noise ratios (Nicola *et al.*, 2004b, 2004a; Nicola *et al.*, 2005) and has an important role in corticostriatal plasticity.

Recently, SNc neurons have been shown to also release GABA in fashion that does not require the vesicular GABA transporter VGAT. Instead, GABA is released

from vesicles exoressing the vesicular monoamine transporter VMAT (Tritsch *et al.*, 2012). However, the wider ramifications of this discovery have yet to emerge.

1.4.3 Subthalamic nucleus

The subthalamic nucleus (STN) is the only glutamatergic body in the basal ganglia and is found dorsal to the SN and ventral to the thalamus, hence its name. STN neurons form a homogenous population of size 10-25 μm with fusiform or polygonal shape (Chang *et al.*, 1983; Kita *et al.*, 1983). They are spontaneously active at 5-15 Hz however this activity is impinged upon by the many sources of afferent input these neurons receive. STN neurons emit 3-4 primary dendrites that arbourise across most of the nucleus (Kita *et al.*, 1983). These dendrites receive glutamatergic input from motor cortex and intralaminar thalamic nuclei which is topographical in nature (Kitai & Deniau, 1981; Afsharpour, 1985; Bevan *et al.*, 1995; Nambu *et al.*, 2002) and dopaminergic input from SNc (Brown *et al.*, 1979; Hassani *et al.*, 1997; Cragg *et al.*, 2004). STN neurons also receive important GABAergic afferents from GPe neurons which are reciprocal with the STN's own excitatory projections to this area (Smith *et al.*, 1990; Shink *et al.*, 1996; Bevan *et al.*, 1997). These GPe-STN connections are important in the context of the indirect pathway as they suppress STN excitation of the output nuclei and thus firing of indirect pathway MSNs results in increased excitation of output nuclei neurons by the STN (Smith & Bolam, 1990a; Shink *et al.*, 1996).

The efferent projections of STN neurons are the only glutamtergic projection of a BG neuron (Smith & Parent, 1988). In addition to the STN-GPe projection, these neurons also project to the BG output nuclei (Nakanishi *et al.*, 1987; Bevan *et al.*, 1994). In particular the cortical-subthalamic-output nuclei pathway has been termed the *hyperdirect pathway* since this is the fastest route that cortical information can be relayed to motor output during movement (Maurice *et al.*, 1998; Nambu *et al.*, 2002; Aron & Poldrack, 2006). Due to the efferent control mediated by the STN, this area is often the target for deep-brain stimulation therapies in the treatment of Prakinson's disease (Anderson *et al.*, 2005; Perlmutter & Mink, 2006).

1.5 Corticostriatal transmission

Whilst cortical projections are topographical in a loose sense, the focal points of these projections exhibit arborisations that are restricted medio-laterally but have a wider spread in the rostro-caudal plane (Gerfen, 1992). Most cortical regions project to both patch and matrix but show a preference for one or the other. For example, layers Vb and VI project mainly to patches whereas layers III-Va project mainly to matrix (Kincaid & Wilson, 1996). Furthermore, individual cortical areas project to multiple striatal foci with these projections being intercalated with similar axonal arborisations from related cortical areas (Flaherty & Graybiel, 1991; Gerfen, 1992; Flaherty & Graybiel, 1994). In this way, individual neostriatal projection neurons or small localized groups of these cells will fire when excited by combinations of inputs from related cortical areas and in doing so encode specific patterns of cortical activity.

1.5.1 Corticostriatal projections and axon terminals

The major target of corticostriatal axons, forming around 90% of corticostriatal synapses, are the heads of the spines of the outer 1/3 of the dendritic arbour of MSNs (Kemp & Powell, 1970; Smith & Bolam, 1990a; Wilson, 2004). Cortical projections to the striatum have been described as having a “cruciform axodendritic arrangement”. This term describes axons that take an extensive course through the tissue, forming synapses with any MSN dendritic spines they encounter. The result is that each MSN receives few synapses from a single cortical neuron and that individual synapses provide weak innervations of MSNs (Wilson & Groves, 1980; Zheng & Wilson, 2002; Wilson, 2004). Anatomical studies have suggested that direct pathway and indirect pathway MSNs receive different inputs from cortical neurons that project via the intratelencephalon compared to those that project only via the descending pyramidal tract. Intratelencepalic neurons had been thought to project mostly or entirely to direct pathway MSN, with pyramidal tract neurons thought to project mostly or entirely to indirect pathway MSNs (Reiner *et al.*, 2003; Lei *et al.*, 2004; Reiner *et al.*, 2010). However a recent optogenetic study suggests that the two cortical projections innervate the two MSN subtypes equally (Kress *et al.*, 2013). Cortical afferents express vesicular glutamate transporter 1 (VGluT1), which distinguishes them from thalamic afferents that express VGluT2 (Kaneko *et al.*, 2002). D1 and D2 MSNs receive the same proportion of cortical/thalamic input (Doig *et al.*, 2010).

1.5.2 AMPA and NMDA receptors

The postsynaptic terminals of cortical projections onto MSNs express 3 types of ionotropic glutamate receptors: AMPA, NMDA and kainate receptors (Cherubini *et al.*, 1988; Stefani *et al.*, 1998; Chergui *et al.*, 2000; Logan *et al.*, 2007; Jeun *et al.*, 2009; Dehorter *et al.*, 2011; Vizcarra-Chacon *et al.*, 2013). At MSN resting Vm, glutamatergic EPSPs are comprised of a large AMPA component and small kainate component , with EPSPs at depolarised potentials or in a Mg^{2+}-free solution producing a significant, long lasting (>1 s) NMDA component (Cherubini *et al.*, 1988; Kita, 1996; Stefani *et al.*, 1998; Logan *et al.*, 2007). AMPA receptors mediate rapid synaptic transmission of the glutamate signal, permitting Na^{+} and K^{+} transmission (and Ca^{2+} in the absence of the GluR2 subunit). AMPA receptor expression at postsynaptic areas is approximately five times the NMDA receptor expression (Jeun *et al.*, 2009). The expressed AMPA receptors are primarily of the GluR2 subunit containing type however GluR3 subunits are preferentially expressed in direct pathway MSNs and GluR1 subunits in indirect pathway neurons (Stefani *et al.*, 1998; Deng *et al.*, 2007; Jeun *et al.*, 2009). NMDA receptors are non-selective with regards to cations but are only open at depolarised potentials due to Mg^{2+} block. The receptor ratio of NR2B/NR2A subunits in NMDA receptors at these synapses has been reported as 0.32±0.03 (Jeun *et al.*, 2009). Postynaptic terminals also express group I mGluRs (Kerner *et al.*, 1997; Smith *et al.*, 2000; Marino & Conn, 2002). Both may be involved in plasiticity (see below) while mGluR5 potentiates NMDA-mediated currents in MSNs (Pisani *et al.*, 2001).

1.5.3 Neurotransmitter release

Presynaptic control of synapses is common throughout the central nervous system (Nicoll *et al.*, 1990; Wu & Saggau, 1997; Miller, 1998). Whether acting as autoreceptors or heteroreceptors, presynaptic receptors may suppress neurotransmitter release by dampening Ca^{2+} dynamics at the presynaptic terminal. Most neurotransmitters (although exceptions exist, e.g. NO) are packaged in intracellular vesicles that must fuse with the plasma membrane in order to exocytose their contents. A simplified explanation of this process is as follows: the vesicle membrane-associated protein synaptobrevin forms a complex with the plasma membrane-associated proteins syntaxin-1A and synaptosomal associated protein (SNAP)-25. These three proteins form a complex which receives α-SNAP,

which in turn receives N-ethylmaleimide-sensitive ATPase (NSF). ATP hydrolysis leads to disassociation of α-SNAP and NSF and partial fusion of vesicular and plasma membranes. At this stage, voltage-gated Ca^{2+} channels form bespoke interactions with the existing complex. The close proximity of these channels facilitates the entry of Ca^{2+} towards this complex and is the final element necessary for complete membrane fusion and neurotransmitter release (DeBello *et al.*, 1995; Martin-Moutot *et al.*, 1996; Matthews, 1996; Zucker, 1996; Stanley, 1997). Disruption of any of these stages (or of local action potentials, which are necessary to open Ca^{2+} channels) will reduce the probability of neurotransmitter release from any single vesicle.

1.5.4 Modulation of neurotransmitter release by GPCRs

Presynaptic G-protein coupled receptors (GPCRs) have been shown on many occasions to suppress neurotransmitter release via a reduction in the activity of N or P/Q type Ca^{2+} channels (Miller, 1990; Hille, 1994; Wu & Saggau, 1997). A direct interaction between the β/γ subunits of the activated G-protein and Ca^{2+} channels is likely to mediate this effect (De Waard *et al.*, 1997; Herlitze *et al.*, 1997; Zamponi *et al.*, 1997; Li *et al.*, 2005).

The presynaptic terminals of corticostriatal synapses express various receptors that have been shown to modulate glutamate release including D_2 (Bamford *et al.*, 2004a; Bamford *et al.*, 2004b), muscarinic (Niittykoski *et al.*, 1999; Pakhotin & Bracci, 2007), μ- and δ- opioid (Jiang & North, 1992; Jose *et al.*, 2007; Blomeley & Bracci, 2011), group III mGluR (Pisani *et al.*, 1997), NK_1 and NK_3 receptors (Blomeley & Bracci, 2008; Blomeley *et al.*, 2009) and A1 (Lovinger & Choi, 1995).

CB1 receptors are expressed presynaptically at corticostriatal synapses and are known to depress glutamate release when activated (Herkenham *et al.*, 1991; Gerdeman & Lovinger, 2001; Adermark *et al.*, 2009). This inhibition occurs through both a reduction in Ca^{2+} transients and through non-Ca^{2+} mechanisms (Gerdeman & Lovinger, 2001). Six NPY receptor subtypes are known to exist, termed Y1 to Y6. NPY receptors and their mRNA are widely expressed in the striatum (Caberlotto *et al.*, 1997; Caberlotto *et al.*, 1998; Caberlotto *et al.*, 2000; Wolak *et al.*, 2003; Stanic *et al.*, 2006). In general NPY is known to mediate presynaptic inhibition in various

brain regions (Colmers *et al.*, 1991; Martire *et al.*, 1995; Qian *et al.*, 1997) however their localization and effects at the synaptic level in the striatum are unknown.

Of course, $GABA_B$ receptors are also implicated in corticostriatal transmission. Although the actions of GABA in the striatum are primarily on postsynaptic $GABA_A$ receptors (Tepper *et al.*, 2004), GABA has also been shown to suppress corticostriatal glutamate release through $GABA_B$ receptors by blocking of Q-type Ca^{2+} channels (Calabresi *et al.*, 1990, 1991; Nisenbaum *et al.*, 1992, 1993; Barral *et al.*, 2000). However whilst the modulation of corticostriatal activity is known, the source of this inhibition by release of endogenous GABA from striatal neurons has not been revealed.

1.5.5 Long term potentiation

Plasticity is the process thorough which molecular changes occur at the synaptic level that result in long term increases or decreases in synaptic strength. Long term potentiation (LTP) is where synaptic strength increases; long term depression (LTD) is the case where synaptic strength decreases. Corticostriatal plasticity is heterogeneous across striatal subregions with LTP more dominant in dorsomedial and rostral regions and LTD more common in dorsolateral and caudal areas. Plasticity is dependent upon high-frequency stimulation (HFS) but whether this is potentiating or depressing is dependent upon receptor activation. LTP is dependent upon HFS, activation of D1, A2A and calmodulin-dependent protein kinase type II (CAMKIIa) and Ca^{2+} influx (Calabresi *et al.*, 2000; Kerr & Wickens, 2001; Lovinger *et al.*, 2003; Shen *et al.*, 2008) and is blocked by muscarinic receptor antagonists (Calabresi *et al.*, 1999a; Lovinger *et al.*, 2003). Upregulation of cAMP production and phosphorylation of DARPP-32 results in increased AMPA receptor expression postsynaptically (Calabresi *et al.*, 2000; Shen *et al.*, 2008). On the other hand striatal LTD functions through a retrograde signalling action. LTD also requires HFS and is blocked by group 1 mGluRs and D1 antagonists (Calabresi *et al.*, 1992a; Calabresi *et al.*, 1992b; Tang *et al.*, 2001; Kreitzer & Malenka, 2005) and requires postsynaptic Cav1.3-type Ca^{2+}-channels (Calabresi *et al.*, 1994). Calcium and metabotropic signals in the postsynaptic neuron converge to trigger endocannabinoid synthesis and release leading to retrograde activation of presynapic CB1 receptors (Gerdeman & Lovinger, 2001; Gerdeman *et al.*, 2002; Kreitzer & Malenka, 2005; Wang *et al.*, 2006; Adermark *et al.*, 2009). CB1 receptor activation and afferent activity alone are sufficient for LTD at these synapses. The

exact intracellular mechanisms are unclear however they are known to incluce cAMP signalling and the active zone protein RIM1α (Adermark & Lovinger, 2007; Chevaleyre *et al.*, 2007; Singla *et al.*, 2007).

NO has also been implicated in corticostriatal plasticity. HFS-dependent LTD can be mimicked by NO donors or blockers of PDEs and can be prevented by antagonists of intracellular signalling proteins such as sGC and cGMP (Calabresi *et al.*, 1999c; Calabresi *et al.*, 2000; Sammut *et al.*, 2010). However NO is also thought to modulate corticostriatal transmission through independent pathways that my work in opposition to the depressive effects of sGC (Sammut *et al.*, 2010).

LTP at the corticostriatal synapse is essential for the regulation of voluntary movement, behavioural control and reward mechanisms. The changes that occur through LTP may therefore underly the cellular basis through which experiences are processed into memory (Calabresi *et al.*, 1996; Wickens *et al.*, 2003).

1.5.6 Short term potentiation

When an action potential invades an axon terminal twice in a short time period of time (<100 ms), the initial AP can modulate release probability in the subsequent AP. This is known as the paired pulse ratio (PPR) (Zucker & Regehr, 2002) When the first AP leads to a build up of Ca^{2+} ions in the presynaptic terminal, the second AP will have more available Ca^{2+} than in the first AP. Since Ca^{2+} is required for vesicular exocytosis, this can result in increased neurotransmitter release in the second spike relative to the first. Such a scenario is referred to as *paired pulse facilitation* (PPF). Similarly, the first AP may deplete the quantity of vesicle in the presynaptic terminal such that when a second AP closely follows, fewer vesicles remain. This can result in reduced neurotransmitter release in the second AP relative to the first. Such a scenario is termed *paired pulse depression* (PPD).

These two processes do not exist in isolation and both are present at most synapses. The PPR is the net difference between these two processes. Corticostriatal synapses exhibit paired pulse facilitation and thalamic synapses exhibit paired pulse depression (Ding *et al.*, 2008),

1.6 Thalamostriatal transmission

The main sources of thalamostriatal projections are the intralaminar nuclei, particularly the centromedian and parafascicular nuclei (Berendse & Groenewegen, 1990; McFarland & Haber, 2000; Erro *et al.*, 2001; Van der Werf *et al.*, 2002; Smith *et al.*, 2004). Most thalamostriatal projections form synapses with the dendritic shafts of MSNs in rats and primates (Smith & Bolam, 1990a; Sadikot *et al.*, 1992; Jakab & Goldman-Rakic, 1996). Striatal MSNs of the direct and indirect pathways receive similar numbers of thalamic afferents however afferents arising specifically from the centromedian nuclei seem to preferentially target direct pathway MSNs (Sidibe & Smith, 1996; Doig *et al.*, 2010). However, whilst thalamic innervation of MSNs is robust, cortical inputs are regarded as playing a more dominant role. Conversely, thalamic inputs are the primary source of excitation in cholinergic interneurons (Lapper & Bolam, 1992; Matsumoto *et al.*, 2001).

Thalamostriatal terminals are known to express several receptors presynaptically including kainate, mGlur1a and Group III metabotropic glutamate receptors and $GABA_B$ receptors (Bradley *et al.*, 1999; Kosinski *et al.*, 1999; Charara *et al.*, 2000; Kieval *et al.*, 2001; Paquet & Smith, 2003). Thalamic terminals also express VGluT2, in contrast with cortical terminals that express VGluT1 (Kaneko *et al.*, 2002).

1.7 GABA receptors

Two classes of GABA receptors exist in the mammalian brain: the fast acting ionotropic $GABA_A$ receptor and the slow acting metabotropic $GABA_B$ receptor. Both receptor types are widespread throughout the striatum.

The $GABA_A$ receptor is a pentameric transmembrane ligand-gated ion channel permeable to Cl^- ions. Thus, at typical physiological ion concentrations, opening of the $GABA_A$ receptor results in influx of Cl^- into the cell and hyperpolarization of the neuron, reducing excitability (Macdonald & Olsen, 1994). The receptor is composed of 5 subunits. Each subunit may be one of several isoforms, which have been termed: 6 α, 3 β, 3 γ and one each of δ, ε, π and θ. At a minimum, a $GABA_A$

receptor must comprise at least one α and one β subunit, with the most common configuration comprising 2 α, 2 β and a single γ subunit (Bormann *et al.*, 1987). The subunit composition of each $GABA_A$ receptor determines the affinity and conductance of the channel. In a typical $GABA_A$ receptor there are 2 GABA binding sites and a benzodiazepine (Bz) binding site. GABA binding sites exist between an α and a β subunit with both sites requiring ligand binding for the channel to open (Connolly *et al.*, 1996). The Bz site is present in most but not all variations of the $GABA_A$ receptor and acts non-competitively in modulatory fashion (Macdonald & Olsen, 1994; Connolly *et al.*, 1996). The single unit conductance of a $GABA_A$ channel can be between 12 and 44 pS, with 30 pS the most common (Bormann *et al.*, 1987). At 30 pS conductance, opening times can vary from 0.5 to 7.6 ms (Bormann *et al.*, 1987). The conductance and opening time of the channel are dependent upon subunit composition and agonist concentration.

$GABA_B$ receptors exert their influence through activation of G-protein coupling and intracellular second-messenger systems (Kaupmann *et al.*, 1997; Padgett & Slesinger, 2010). The $GABA_B$ receptor G-protein is of the Gi/o type and mediates its effects through direct interactions with ion channels and by inhibition of adenylyl cyclise and hence reducing cAMP production (Padgett & Slesinger, 2010). $GABA_B$ receptors are hetrodimers of two subunits, $GABA_{B1}$ and $GABA_{B2}$ and both subunits are necessary for the receptor to function (Kaupmann *et al.*, 1997; Jones *et al.*, 1998; Prosser *et al.*, 2001). The $GABA_{B1}$ subunit binds directly with extracellular GABA whereas the $GABA_{B2}$ subunit is necessary for trafficking of the receptor complex and activation of the G-protein (Galvez *et al.*, 2000; Jones *et al.*, 2000; Calver *et al.*, 2001). The C-terminal domain of the $GABA_{B2}$ subunit is is essential to the receptor surface trafficking process (Calver *et al.*, 2001). Presynaptically, $GABA_B$ receptors inhibit neurotransmitter release through reduced opening of voltage gated Ca^{2+} channels and this has been shown in striatal synapses (Barral *et al.*, 2000). Postsynaptically, $GABA_B$ receptors are coupled to inwardly rectifying K^+ channels and therefore hyperpolarise the postsynaptic neuron by reducing activity of these channels (Calver *et al.*, 2002).

1.8 Action selection

Generally, action selection is the process by which a control system selects from competing interests for use of finite resources. The problem of action selection is one that is most commonly applied to vertebrate systems and to robots, regarding how these entities decide upon the most appropriate or beneficial movement (or series of movements) from many possibilities.

The macrocircuitry of the basal ganglia appear highly configured for action selection by integrating information on internal body state and external stimuli. The striatum in particular receives connections from cortex, thalamus and midbrain dopaminergic neurons (Parent & Hazrati, 1995; Haber *et al.*, 2000; McFarland & Haber, 2000). Striatal MSNs of the direct and indirect pathways that are subject to these inputs can facilitate movement or cessate movement upon firing (Albin *et al.*, 1989; DeLong, 1990; Smith *et al.*, 1998; Kravitz *et al.*, 2010). However, whilst individual striatal neurons receive 10^4 cortical synapses (Wilson *et al.*, 1983; Ingham *et al.*, 1993), a high convergence of excitatory input onto these neurons is required for MSN firing. This provides an architecture where weakly correlated or less salient inputs are filtered out in favour of those which are more important.

Furthermore, cortical projections to the striatum are not uniform; they are arranged based upon topography (eg rostrocaudal/ventromedial) and function (eg associative/sensory/motor), with individual projections forming groups of synapses in a limited area. Therefore striatal neurons that participate in the same function or are spatially close are more likely to receive the same input, whereas neurons that evoke movement responses based upon differing criteria or are far away from each other are less likely to receive afferent input from the same cortical neurons (Selemon & Goldman-Rakic, 1985; Flaherty & Graybiel, 1991; Kincaid *et al.*, 1998).

This linking of neurons with similar function and topography is not exclusive to the corticostriatal projection, but is also expressed to some degree in MSN projections to output nuclei (DeLong *et al.*, 1985; Hamada *et al.*, 1990; Hazrati & Parent, 1992). The output nuclei pass this information onto thalamic nuclei (Kuo & Carpenter, 1973; Kim *et al.*, 1976; Gerfen *et al.*, 1982; Deniau & Chevalier, 1992; Ilinsky *et al.*, 1997) which in turn project back to cortical regions that provided the original input to the striatum (Alexander *et al.*, 1986). Thus, a series of functionally distinct

cortico-basal ganglia-thalamocortical loops, passing though the striatum, form the basis of our understanding of physiological models of action selection (Alexander *et al.*, 1986).

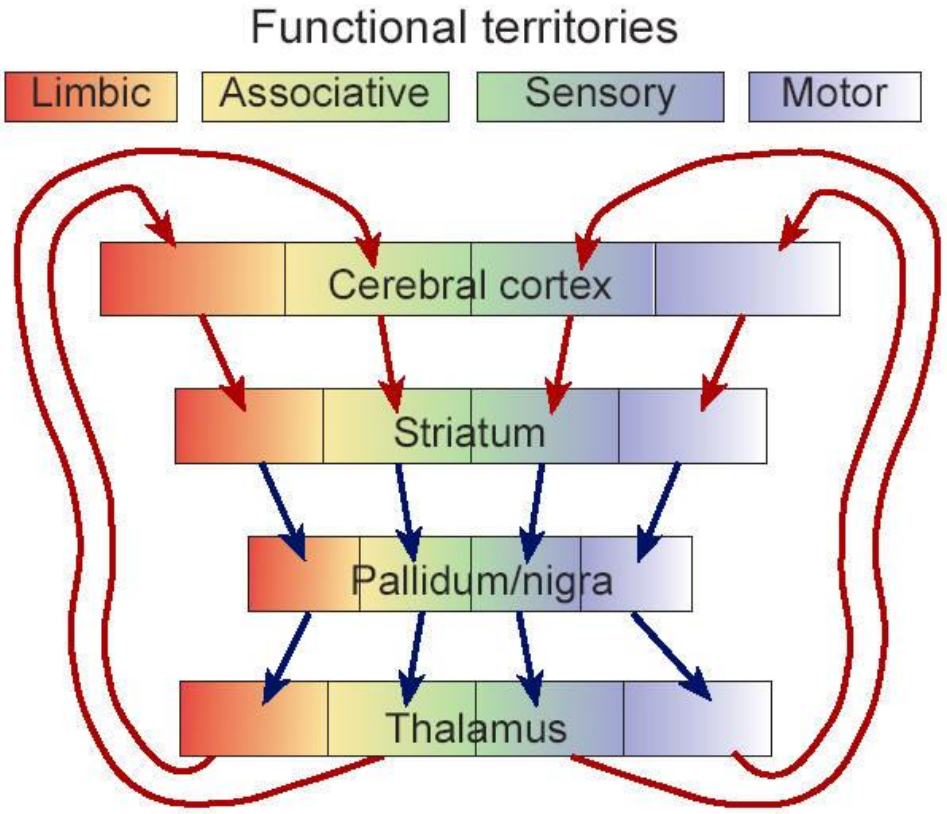

Figure 1.2: Cortical loops are functionally segregated. Adapted from Redgrave (2007). Cortical areas responsible for differing functionality project to the striatum and maintain this functional segregation throughout a series of loops that loop back to the original cortical region. However, at each station in the respective loops, other neurons in these areas outwith the respective loop can modulate the activity and progression of the loop.

Of course, projection neurons of the striatum and output nuclei do not only project to the next stage in the loop, but also form lateral synapses with other projection neurons. GP neurons innervate nearby GP neurons (Kita & Kitai, 1994; Nambu & Llinas, 1997; Bevan *et al.*, 1998; Sato *et al.*, 2000) and MSNs modulate nearby MSNs both directly (Wilson & Groves, 1980; Kawaguchi *et al.*, 1995; Tunstall *et al.*, 2002; Plenz, 2003) and through presynaptic modulation of corticostriatal projections (Blomeley *et al.*, 2009; Blomeley & Bracci, 2011).

This has led to the creation of the winner-take-all theory (Groves, 1983), where cortical input converges upon the striatum which selects a single winner. Phasic cortical inputs represent “bids” or “actions” by depolarising striatal neurons until enough salient input results in a “winning” MSN that not only mediates a motor response but also suppresses neighbouring MSNs through lateral connections and in doing so consolidating its winning status. Thus while functionally each loop may be relatively segregated, physiologically their lateral interactions are important in the selection process.

Many further theories on basal ganglia function alter or build upon this basic model of action selection of interaction between parallel cortical loops (Mink, 1996; Fukai & Tanaka, 1997; Berns & Sejnowski, 1998; Redgrave *et al.*, 1999; Bar-Gad *et al.*, 2003; Gurney *et al.*, 2004). Furthermore a series of phylogenetically older *sub*cortical loops, which relay information via the superior colliculus, periaqueductal grey matter and pendunculopontine nucleus, have also been proposed (McHaffie *et al.*, 2005).

1.9 Striatal neurons

The striatum expresses several discrete neuron types. Overwhelmingly, the striatum is populated by the projection neurons, the medium spiny neurons, which are GABAergic. Classically, three interneuron types were known to exist: the fast spiking interneuron and low threshold spiking interneuron are GABAergic; the cholinergic interneuron primarily releases acetylcholine. More recently, several other neuron types have been discovered. Each of these is detailed below.

1.9.1 Principal neurons: the medium spiny neuron

MSNs are the principal cells of the striatum and represent more than 95% of all striatal neurons in rodents with a greater proportion of interneurons in primates (Kemp & Powell, 1971; Wu & Parent, 2000). MSN somata are medium sized, being approximately 12-25 µm in diameter, and MSN dendrites exhibit dense spiny projections (DiFiglia *et al.*, 1976; Wilson & Groves, 1980). MSN dendritic arbours

are 300-500 µm in diameter and do not cross the patch/matrix boundary (Wilson & Groves, 1980; Malach & Graybiel, 1986).

MSNs receive their input from across the cortex and from thalamic intralaminar nuclei. 85% of striatal synapses derive from cortico-thalamic input and 90% of cortical synapses in the striatum synapse onto MSNs (Wilson, 2004). Each MSN receives only one or two synapses from a single cortical neuron (Wilson, 2004). Therefore, with each MSN possessing dendritic spines of the order of 15,000 in number, a huge convergence of inputs at the level of a single MSN is implied. In this fashion, a single MSN can only be depolarised when there is sufficient near-simultaneous excitation from multiple converging cortical neurons (Stern *et al.*, 1997; Stern *et al.*, 1998).

All MSNs project both outside the striatum and intrastriatally. The primary axon projections differ depending upon the two MSN subtypes: striatonigral MSNs project primarily to the SNr and GPi/EPN (but also to the GPi) whereas striatopallidal MSNs project almost exclusively to the GP (Kawaguchi *et al.*, 1989; DeLong, 1990; Kawaguchi *et al.*, 1990; Albin *et al.*, 1995; Kawaguchi, 1997; Smith *et al.*, 1998; Tunstall *et al.*, 2002; Chuhma *et al.*, 2011). MSNs also project to each other. These parallel MSN-MSN synapses exist at relatively distant locations on the dendritic arbour and thus the direct $GABA_A$ receptor-mediated current is relatively weak (Wilson & Groves, 1980; Somogyi *et al.*, 1981; Kawaguchi *et al.*, 1995; Tunstall *et al.*, 2002; Plenz, 2003). Striatonigral MSNs readily synapse with striatopallidal MSNs and vice versa (Yung *et al.*, 1996). MSNs also project onto THIs (Ibanez-Sandoval *et al.*, 2010). MSNs also receive synaptic input from interneurons (Tepper & Bolam, 2004) and collaterals from other MSNs (Bouyer *et al.*, 1984).

Figure 1.3 illustrates the electrophysiological properties of MSNs (Wilson & Groves, 1981; Nisenbaum & Wilson, 1995). A distinctive property of MSNs is their fast inwardly rectifying potassium channels (I_{KIR}). I_{KIR} is activated at hyperpolarised potentials and is the dominant factor contributing to the low input resistance of MSNs (Kita *et al.*, 1984). Although permeable to K^+, they are preferentially activated at hyperpolarised potentials which, in contrast to conventional voltage gated potassium channels, mediate inward current that depolarises the membrane. Since these channels are open at rest, they contribute to a low input resistance. When MSNs are hyperpolarised, positive current through these channels increases

as deviation from the K^+ reversal potential increases, thus fulfilling their "inwardly rectifying" properties. At potentials above -60 mV, I_{KIR} is inactivated and input resistance increase (Nisenbaum *et al.*, 1996).

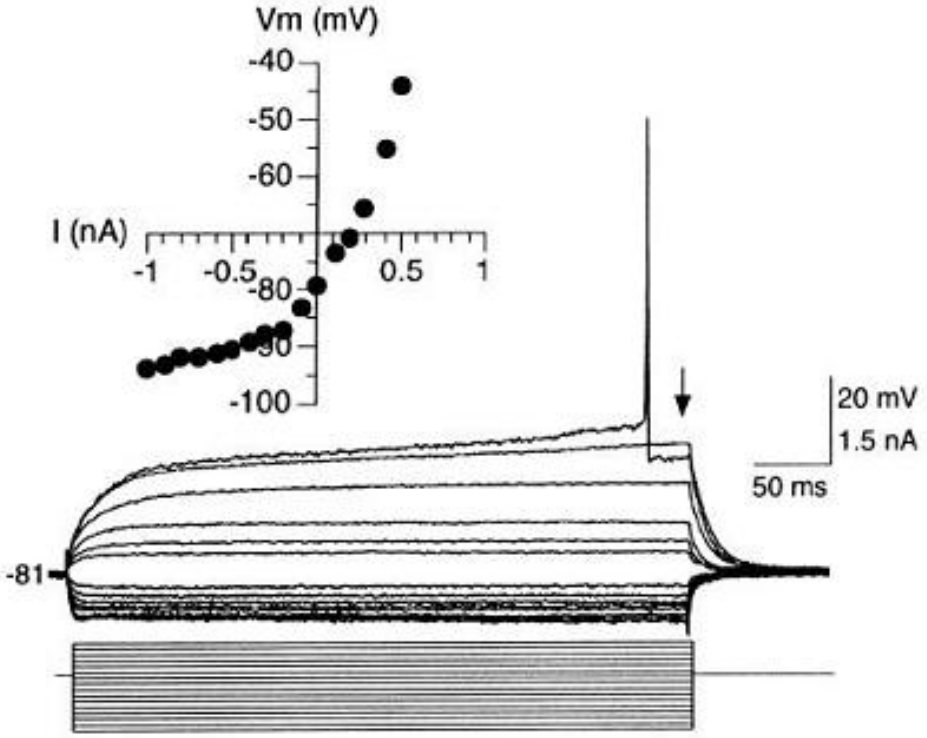

Figure 1.3: Electrophysiological characteristics of MSNs. Responses of an MSN to to depolarising (0.1-.06 nA) and hyperpolarising (0.1-1.0 nA) current injection of 400 ms duration. *Inset:* I-V plot for the responses shown in the main figure. Each point is the membrane potential after 390 ms (denoted by an arrow) to constant current injection. The defining characteristics of MSNs are a low resting membrane potential (-80 to -90mV), low input resistance and a slow depolarisation to initial spike followed by regular spiking.. Adapted from Nisenbaum & Wilson (1995).

MSNs exhibit a characteristic "slow depolarisation" before spiking. At least three K^+ conductances respond to depolarising currents by acting as a "brake" upon conventional voltage gated Na^+ currents. These are the fast- and slow- inactivating potassium currents (I_{Af} and I_{As} respectively) and the persistent potassium current, which differ in their voltage dependence and inactivation kinetics (Surmeier *et al.*, 1989; Nisenbaum *et al.*, 1996; Tkatch *et al.*, 2000; Shen *et al.*, 2004). I_{Af} and I_{As} are both transiently active and activate upon depolarisation, with I_{Af} inactivating in the

tens of milliseconds range and I_{As} in the hundreds of milliseconds range. As these rectifying currents inactivate, input resistance increases causing further depolarisation in response to the same magnitude of current. Therefore, the neuron becomes more excitable. The persistent sodium current either does not inactivate or only inactivates after several seconds.

MSNs express both I_{BK} and I_{SK} potassium channels. These are dependent upon Ca^{2+} ion activation and mediate large (I_{BK}) and small (I_{SK}) K^{+} conductances. NMDA-dependent Ca^{2+} entry during spiking activates these channels and mediates large and small afterhyperpolarisations (Bargas *et al.*, 1999). MSNs are also subject to inward and outward rectification. Inward rectification is where hyperpolarisation of a neuron triggers a homeostatic change in channels that return the neurons to its resting membrane potential. Similarly, outward rectification is the process when the neuron is depolarised. In MSNs, inward rectification via sodium and calcium currents and outward rectification due to slowly non-activating potassium currents (Galarraga *et al.*, 1994; Nisenbaum *et al.*, 1994; Nisenbaum & Wilson, 1995; Bargas *et al.*, 1999).

Whilst MSNs are generally considered a single class of neuron, differences exist between intrinsic properties of channels between MSNs of the striatonigral and striatopallidal type. These distinct properties manifest as subtle differences in excitability and pharmacology. The signature difference between the two types is dopamine receptor expression, with D1 receptors expressed almost exclusively by striatonigral MSNs and D2 by striatopallidal neurons (Gerfen *et al.*, 1990; Surmeier *et al.*, 1996; Surmeier *et al.*, 2007). D1 receptor activation reduces sodium channel opening (Schiffmann *et al.*, 1995) and enhances I_{KIR} (Pacheco-Cano *et al.*, 1996) thus reducing excitability. However they also enhance firing through activating L-type Ca^{2+} currents and blocking Kv channels (Hernandez-Lopez *et al.*, 1997; Nisenbaum *et al.*, 1998). Conversely, D2 channels reduce I_{KIR} (Uchimura & North, 1990) and inhibit L-type Ca^{2+} channels (Hernandez-Lopez *et al.*, 2000). Striatonigral MSNs exhibit muscarinic M4 receptors and release substance P and dynorphin, while striatopallidal neurons express adenosine A2A receptors and release enkephalin (Gerfen, 1992; Ince *et al.*, 1997; Bolam *et al.*, 2000). MSNs of both types express M1 receptors, which inhibit N- and P/Q-type Ca^{2+} channels that are coupled to Ca^{2+}-activated potassium currents and lower the activation potentials of A-type potassium currents (Akins *et al.*, 1990; Calabresi *et al.*, 1999b).

1.9.2 Cholinergic interneuron

Cholinergic interneurons, also referred to as large aspiny interneurons (LAIs) or tonically active neurons (TANs), are the major non-GABAergic striatal neuron. LAIs express several markers of acetylcholine (ACh) including immunopositivity for choline acetyltransferase (ChAT), acetylcholinetsterase (AChE) and the vesicular ACh transporter (VAChT) (Zhou *et al.*, 2002). This cholinergic activity is the defining feature of these neurons along with their conspicuous size, with a somatic diameter of ~40µm in comparison to other striatal neurons that are closer to 20 µm (Bolam *et al.*, 1984). Cholinergic interneurons comprise a mere 1-2% of striatal neurons (Kemp & Powell, 1971; Rymar *et al.*, 2004). Cholinergic interneurons emit 3-6 spineless dendrites that exhibit few branches (Wilson *et al.*, 1990) and extend up to 1 mm in the rosto-caudal plane and 500 µm in the dorso-ventral and medio-lateral and widespread axonal arborisations that synapse predominantly onto MSNs but also FSIs (Izzo & Bolam, 1988; Chang & Kita, 1992; Bennett & Wilson, 1999; Zhou *et al.*, 2002). In contrast to other striatal neurons, LAIs receive excitatory input almost exclusively from thalamic nuclei (Meredith & Wouterlood, 1990; Lapper & Bolam, 1992; Thomas *et al.*, 2000). Cholinergic interneurons also receive inhibitory synapses from MSNs and dopaminergic input from SNr (Bolam & Bennet, 1995; Thomas *et al.*, 2000).

Thalamic and dopaminergic inputs converge to elicit a burst-pause response in cholinergic interneurons during reward and predicted reward (Graybiel *et al.*, 1994; Morris *et al.*, 2004). These burst-pause responses suppress cortical inputs onto MSNs of both subtypes and increase postsynaptic responsiveness in striatopallidal MSNs (Ding *et al.*, 2010). LAIs also mediate effects upon MSNs disynaptically. LAIs mediate nAChR-mediated GABAergic inhibition of MSNs via THIs and NGFIs (English *et al.*, 2011; Luo *et al.*, 2013). Nicotinic ACh receptors are also expressed on dopaminergic nerve terminals in the striatum and modulate dopamine release (Jones *et al.*, 2001; Zhou *et al.*, 2001).

The electrophysiologicl properties of cholinergic interneurons are shown in Figure 1.4. Cholinergic interneurons are characterised electrophysiologically by their high input resistance, depolarised resting Vm and spontaneous activity both *in vivo* and *in vitro* (Kawaguchi, 1993; Bennett & Wilson, 1999), some of which fire in a simple constant pattern, otherwise which rhythmically oscillate between bursts and

periods of quiescence. Their firing rate is limited to 2-10 Hz (Wilson *et al.*, 1990; Wilson & Goldberg, 2006)

At any subthreshold membrane potential, the intrinsic properties of the membrane will lead the cell to depolarise (Kimura *et al.*, 1984; Aosaki *et al.*, 1995; Bennett & Wilson, 1999; Bennett *et al.*, 2000; Zhou *et al.*, 2002). At -65mV, a non-inactivating "persistent" Na^+ current opens ($I_{Na,P}$), depolarising the cell. This depolarisation increases activation of $I_{Na,P}$ and thus the current exerts positive feedback upon the cell. At spike threshold (-50mV), the cell depolarises and influx of calcium occurs through activated calcium channels. These channels inactivate slowly, therefore the constant calcium current prolongs the action potential somewhat whilst activating the I_{AHP}, the calcium-dependent potassium current. $I_{Na,P}$ is inactivated upon the hyperpolarisation mediated by I_{AHP}, which also activates I_H, a cation channel that begins to mediate depolarisation. The cell continues to depolarize due to Ca^{2+} influx inactivating the I_{AHP} and due to I_H until the Vm reaches approximately -60mV, where I_H is inactivated and $I_{Na,P}$ becomes active once more. From here the cycle restarts.

Cholinergic interneurons also express I_{KIR} channels that are activated when the cell is hyperpolarised, similar to MSNs (Wilson & Goldberg, 2006). In this case, however, I_{KIR} activation further hyperpolarises the cell due to the resting Vm of LAIs being less negative than the reversal potential for K^+ ions, unlike in MSNs.

Cholinergic interneurons express D2 receptors, which depolarise the neuron through cAMP-mediated mechanisms (Aosaki *et al.*, 1994), and D5 receptors, which inhibit sodium channels (Maurice *et al.*, 2004). D2 receptors have higher affinity for dopamine than D5 receptors; therefore tonic dopamine generally produces depolarising responses, except where there are spikes in dopaminergic input when both D2 and D5 receptors are activated. LAIs express muscarinic M2 and M4 receptors, both of which exert autoinhibition through activation of K^+ channels (Calabresi *et al.*, 1998). Cholinergic interneurons also respond to serotoninergic input. 5HT-2 receptors are expressed on LAIs; upon activation, LAIs are excited by inactivation of Ca^{2+} dependent AHPs (Blomeley & Bracci, 2005). Finally, LAIs are also directly inhibited by μ-opioid agonists (Ponterio *et al.*, 2013).

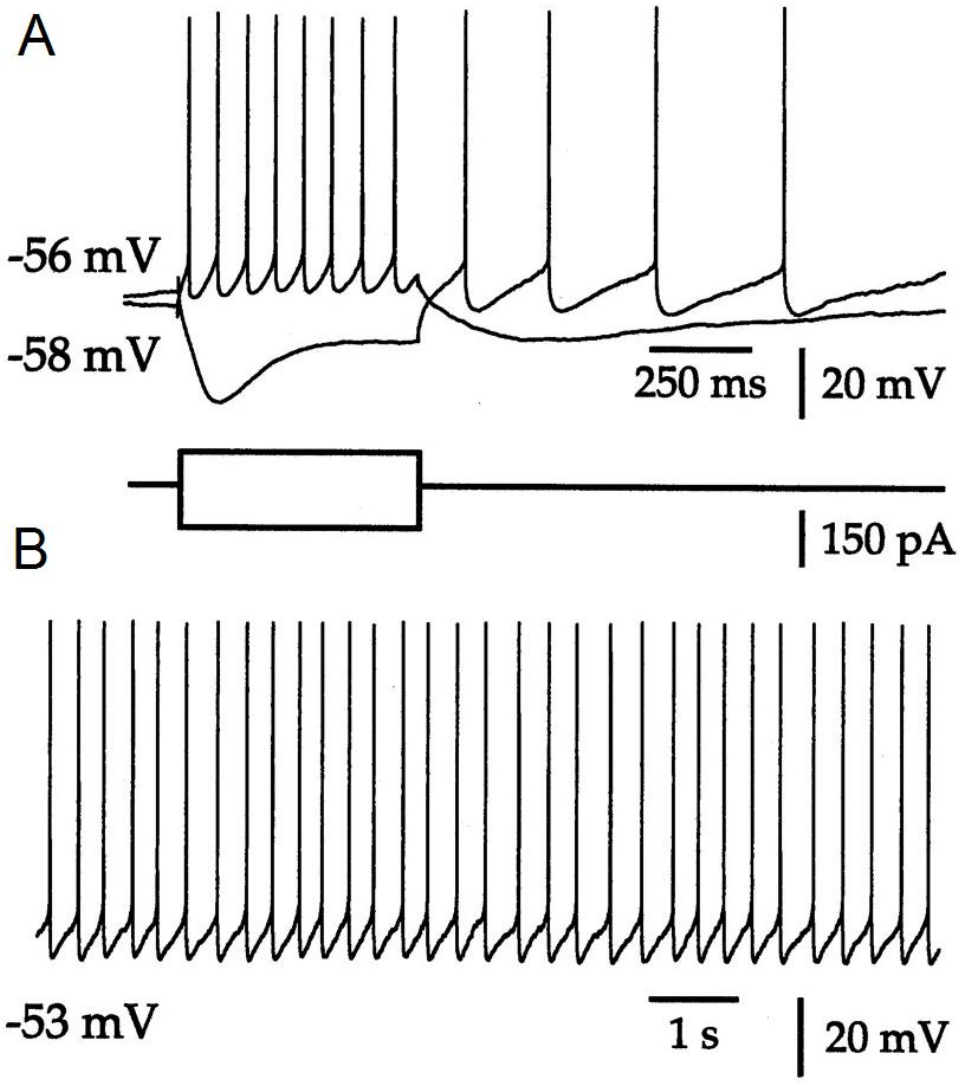

Figure 1.4: Electrophysiological characteristics of LAIs. (**A)** Response of an LAI having undergone whole-cell patch clamp to positive and negative current steps. **(B)** Recording from the same neuron in the absence of any current injection. LAIs are characterised by spontaneous activity, large AHPs with a pronounced sag and rebound spikes after hyperpolarisation. Adapted from Bennett & Wilson (1999).

1.9.3 Fast spiking interneuron

Fast spiking interneurons (FSIs) were first characterised as the parvalbumin expressing interneurons of the striatum (Cowan *et al.*, 1990) and comprise ~0.7% of rat striatal neurons (Luk & Sadikot, 2001). Their expression in the striatum is not heterogeneous; FSIs are more prevalent in the dorsolateral area of the striatum (Kita *et al.*, 1990), however they are expressed to some degree in all areas. They are similar to other parvalbumin expressing fast spiking cells of the hippocampus and cortex (Wilson, 2004) that also have short action potential duration and high firing rate. FSIs feature a spherical soma of 10-30 µm diameter with aspiny dendrites with varied arbourisations of 100-600 µm (Kita *et al.*, 1990). FSI dendrites are electrotonically coupled through gap junctions, with one third of tested FSI pairs exhibiting coupling (Koos & Tepper, 1999; Koos *et al.*, 2004). These gap junctions permit transfer of ions and may entrain a degree of synchrony amongst local FSI populations. FSIs receive excitatory glutamatergic input from the cortex and thalamus (Lapper *et al.*, 1992; Sidibe & Smith, 1999; Ramanathan *et al.*, 2002) and receive inhibitory inputs from cholinergic interneurons and pallidal neurons (Chang & Kita, 1992; Bevan *et al.*, 1998). Glutamatergic inputs do not feature any NMDA-receptor mediated currents (Gittis *et al.*, 2010). In contrast to MSNs, FSIs receive many synaptic contacts from each afferent axon and thus may generate spikes from a level of synchronous activity much lower than that required in MSNs (Ramanathan *et al.*, 2002). When cortical input into the striatum is desynchronised, FSIs are preferentially activated over MSNs due to a lower requirement for simultaneous input. The inhibitory tone and concurrent decrease in converging input onto MSNs can lead to a pronounced reduction of MSN activity (Mallet *et al.*, 2005).

FSIs principally target MSNs, where they form synapses more proximally to the soma than LTSIs and other MSNs (Kubota & Kawaguchi, 2000; Gittis *et al.*, 2010; Planert *et al.*, 2010). The inhibitory input is thus relatively stronger for FSIs than these other neurons, such that bursts even in a single FSI can delay spiking in MSNs (Koos & Tepper, 1999). FSIs preferentially target MSNs of the direct pathway over the indirect pathway (Gittis *et al.*, 2010), however both receive significant inhibition, with individual FSIs innervating MSNs of each type (Planert *et al.*, 2010). Single FSIs are known to innervate over 100 MSNs (Koos & Tepper, 1999), thus with FSIs exerting powerful feedforward inhibition between

corticostriatal projections and MSNs (Bennett & Bolam, 1994; Kawaguchi & Kubota, 1997), a powerful control of striatal circuits is implied.

In-vitro, FSIs are characterised electrophysiologically by a short action potential duration, short but deep afterhyperpolarisation and very high firing rates of up to 200 Hz (Kawaguchi, 1993; Koos & Tepper, 1999). These characteristics are depicted in Figure 1.5. During suprathreshold current injection, periods of quiescence between bursts of spikes exhibit membrane oscillations that are intrinsic and dependent on voltage gated sodium channels (Koos & Tepper, 1999; Bracci *et al.*, 2003) however the precise mechanisms require elucidation. Such channels trigger trains of action potentials that may be responsible for patterns of firing *in vivo* and *in vitro* (Bracci *et al.*, 2003).

FSIs respond to modulation by dopamine, ACh and serotonin. Dopamine depolarises FSIs via excitatory D5 receptors and D2 mediated inhibition of GABAergic afferents (Bracci *et al.*, 2002; Centonze *et al.*, 2003). FSIs express nicotinic receptors which depolarise upon ACh binding; ACh may also depolarise FSIs indirectly by raising dopaminergic tone through activation of nigrostriatal pathways (Chang & Kita, 1992; Koos & Tepper, 2002; Zhou *et al.*, 2002). Finally, FSIs express 5-HT_{2C} receptors which upon activation increase excitability of FSIs by inactivation of inwardly rectifying potassium channels (Blomeley & Bracci, 2009).

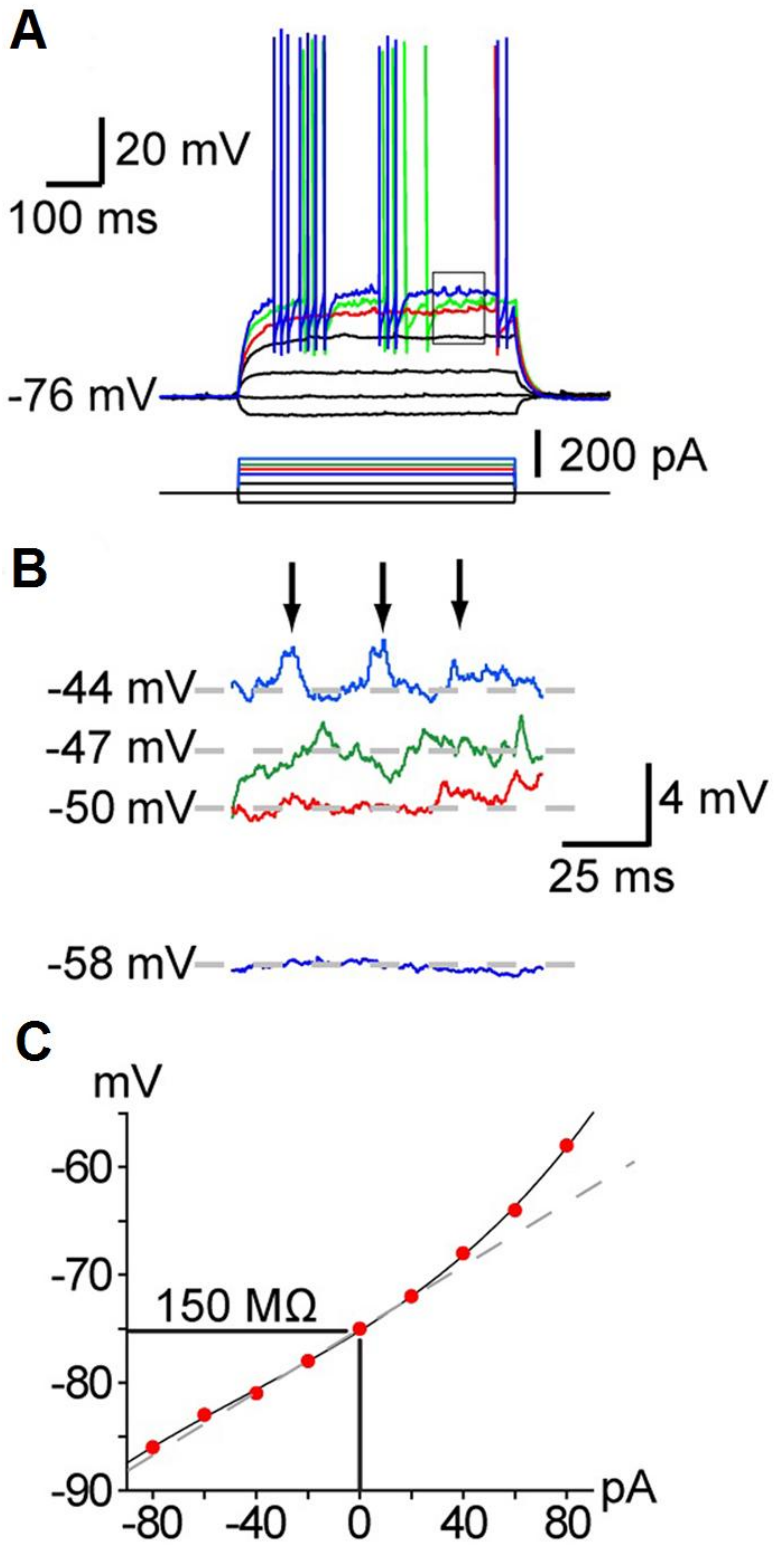

Figure 1.5: Electrophysiological characteristics of FSIs. (A) Response of a patched FSI to current injection. **(B)** Expanded view of the rectangle in (A) showing subthreshold membrane oscillations in response to current injection. **(C)** I/V curve of the neuron shown in (A). FSIs are defined by very short action potential durations, high rate of fire (up to 200Hz) burst activity and membrane oscillations at depolarised potentials. Adapted from Tepper *et al.* (2010).

1.9.4 Low threshold spiking interneuron

Low threshold spiking interneurons (LTSIs) are less well characterised than MSNs, FSIs or LAIs. They were first characterised by expression of somatostatin (SSN), neuropeptide Y, nitric oxide (NO) synthase and NADPH diaphorase (Vincent *et al.*, 1983; Chesselet & Graybiel, 1986; Smith & Parent, 1986; Figueredo-Cardenas *et al.*, 1996; Partridge *et al.*, 2009) and account for 0.8% of rat striatal neurons in rats (Rymar *et al.*, 2004). LTSIs populate all striatal areas but are most densely expressed in dorsomedial regions (Gerfen *et al.*, 1985). LTSIs have medium sized, fusiform and spherical somata with diameters of 9-25 µm. LTSIs emit 3-5 thick, aspiny dendrites with few branches that extend up to 600 µm (DiFiglia & Aronin, 1982; Vincent & Johansson, 1983; Kawaguchi, 1993). Compared to other striatal neurons, axonal arbourisations are the least dense but have the greatest extent, with axons of few branches extending up to 1 mm (Kawaguchi, 1993; Kubota & Kawaguchi, 2000). LTSIs receive glutamatergic input from cortex and thalamus (Vuillet *et al.*, 1989a; Vuillet *et al.*, 1989b; Sidibe & Smith, 1999) with these inputs relatively weak compared to similar inputs onto MSNs (Partridge *et al.*, 2009; Gittis *et al.*, 2010). Neurons of the GP also form GABAergic synapses with LTSIs (Bevan *et al.*, 1998).

LTSIs form synapses on the distal dendrites of MSNs, with weak or undetectable GABAergic IPSPs (DiFiglia & Aronin, 1982; Aoki & Pickel, 1988; Vuillet *et al.*, 1989a; Vuillet *et al.*, 1989b; Kubota & Kawaguchi, 2000; Gittis *et al.*, 2010). As the GABA-mediated effects of LTSIs are weak, it is believed that the primary role of these neurons is modulatory, through the release of NO, SSN and/or NPY. SSN has been shown to presynaptically inhibit GABA release at MSN-MSN synapses (Lopez-Huerta *et al.*, 2008).

NO released from LTSIs is known to modulate MSN activity and likely has a role in plasticity, LTSI axon projections that terminate on MSN dendrites contain neuronal nitric oxide synthase (nNOS), an enzyme necessary for NO production (Calabresi *et al.*, 1999c; Sancesario *et al.*, 2000). Further, the shafts of the dendritic spines where these axon projections terminate contain signalling proteins associated with NO including soluble guanylate cyclise (sGC), cyclic guanosine monophosphate (cGMP), cGMP-dependent protein kinase and inhibitors of these proteins, the phosphodiesterases (PDEs) (Ariano *et al.*, 1982; Matsuoka *et al.*, 1992; Fujishige *et al.*, 1999; Ding *et al.*, 2004). Intrastriatal infusion of cGMP analogues and local

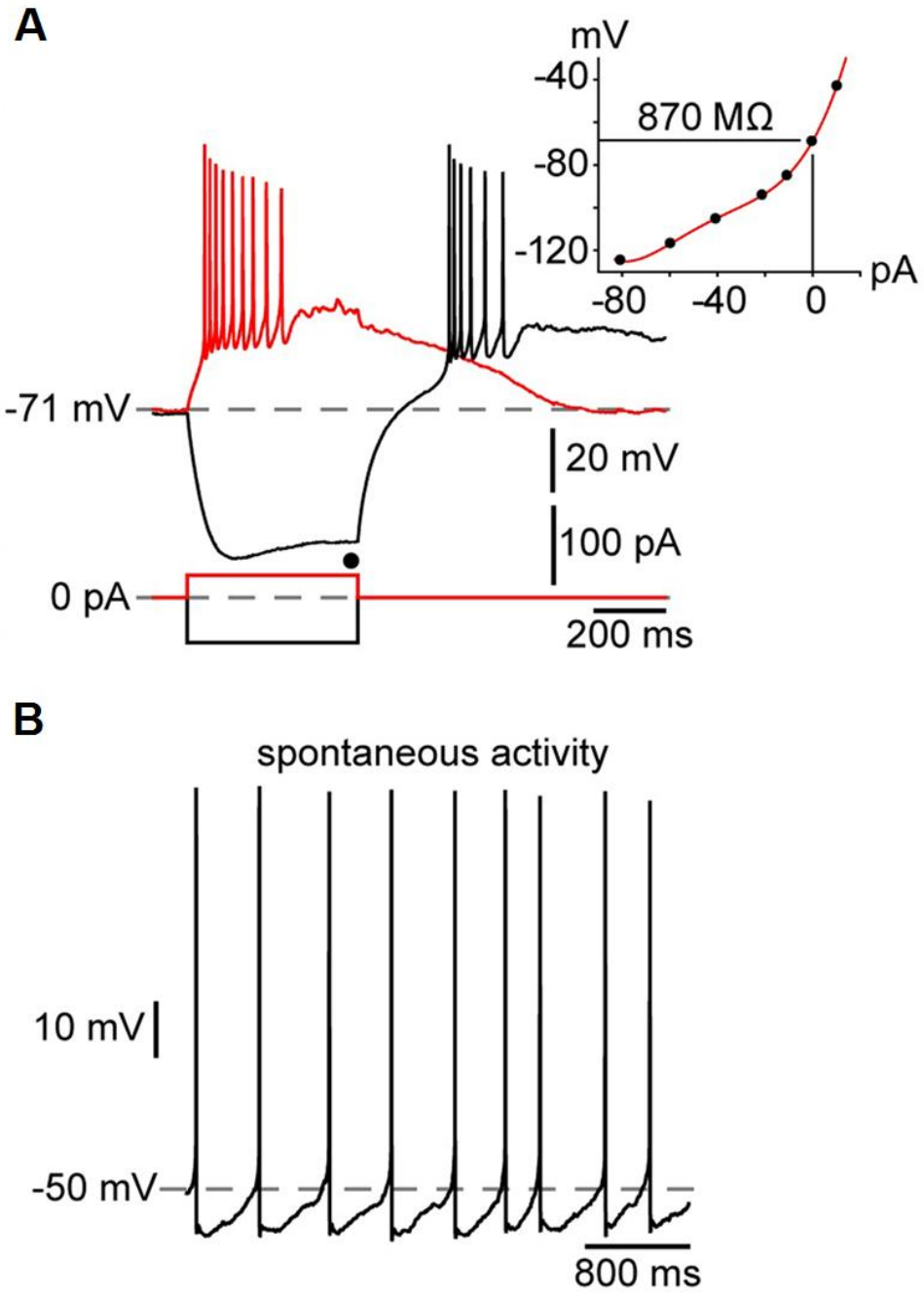

Figure 1.6: Electrophysiological characteristics of LTSIs. (A) Properties of a patched LTSI in response to depolarising current injection from rest (red trace) and the response to cessation of hyperpolarising current injection (black trace). *Inset:* I/V curve of neuron shown in main panel. **(B)** Spontaneous activity in a different LTSI. LTSIs are defined by a high resting Vm, high input resistance, low threshold for spiking, rebound depolarisations after sustained hyperpolarisation and Ca^{2+} dependant plateau after depolarisation. Adapted from Tepper *et al.* (2010),

administration of PDE inhibitors have each been shown to depolarise MSNs (West & Grace, 2004; Threlfell *et al.*, 2009). The effects of NO on plasticity are discussed in section 1.5.5.

LTS interneurons are characterised electrophysiologically by their high input resistances (>600 MΩ), depolarised resting Vm (-60 to -50 mV), low threshold for spiking, Ca^{2+}-dependent spikes with concomitant longer action potential durations and plateau potentials (Kawaguchi, 1993; Kubota & Kawaguchi, 2000; Centonze *et al.*, 2002; Ibanez-Sandoval *et al.*, 2010). Both spikes and plateaus are mediated by slow influx of Ca^{2+} ions. LTSIs also exhibit rebound depolarisations: following sustained hyperpolarisation, LTSIs will depolarise above resting Vm when a hyperpolarising current ends. *In-vitro*, LTSIs can be spontaneously active in an asynchronous manner (Partridge *et al.*, 2009).

LTSIs express D1 and D5 dopamine receptors, which depolarise LTS neurons and lead to significant increases in firing rate (Centonze *et al.*, 2002; Rivera *et al.*, 2002) although the signalling mechanisms involved are unknown. M1 and M2 receptors are also expressed but their roles are also to be determined (Bernard *et al.*, 1998). LTSIs are inhibited by serotonin (Cains *et al.*, 2012).

1.9.5 Neurogliaform interneuron

Neurogliaform interneurons (NGFIs) have been known to exist in striatum for some time (Dimova *et al.*, 1980; Sancesario *et al.*, 1998; Rodriguez-Pallares *et al.*, 2000) however have only recently been characterised in detail. Like LTSIs, are immunopositive for NPY but, unlike low-threshold neurons, not for NOS or SSN. They are similar to NPY-expressing neurons of the cortex and hippocampus (Karagiannis *et al.*, 2009; Karayannis *et al.*, 2010) and comprise around 0.19% of striatal neurons in mice (Ibanez-Sandoval *et al.*, 2011). Soma size of these neurons is 11-15 µm. They emit five to nine primary dendrites that branch 20-30 µm from the soma and form small but dense dendritic arbours 150-280 µm diameter with few spines. The NGF axonal network is also dense and highly branched, projecting radially up to 800 µm but especially dense between 100 and 200 µm radii (Ibanez-Sandoval *et al.*, 2011). These neurons are excited by glutamatergic cortical input and by cholinergic interneurons via nicotinic ACh receptors (English *et al.*, 2011; Ibanez-Sandoval *et al.*, 2011). NGFIs elicit $GABA_A$-

mediated inhibition of MSNs, activity that can be directed by the cholinergic interneuron burst-pause response (English *et al.*, 2011; Luo *et al.*, 2013). Figure 1.7 shows the electrophysiological properties of NGFIs.

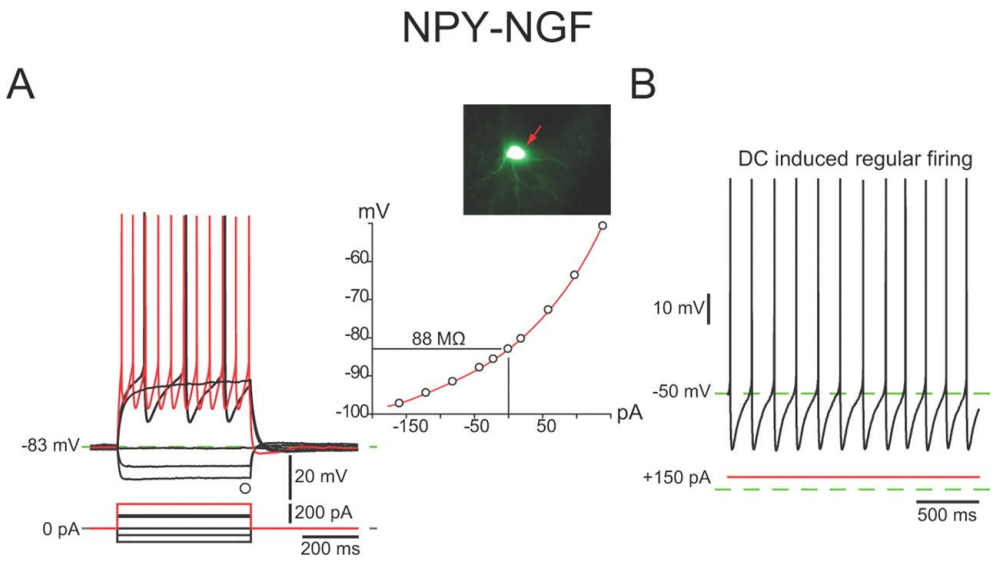

Figure 1.7: Electrophysiological characterisitcs of NGFIs. NGFIs are characterised by low input resistance, low resting Vm, large AHPs and lack of spike accommodation. **(A)** Current-clamp recording of NGFI showing responses to positive and negative current injections. Low input resistance and low resting Vm are apparent. Inset, inward rectification exists at both hyperpolarised and depolarised potentials. **(B)** In the absence of current injection, NGFIs do not fire but produce regular spiking upon depolarising current injection. Adapted from Ibanez-Sandanoval *et al*. (2011).

1.9.6 Tyrosine hydroxylase-positive interneuron

Whilst a proportion of striatal neurons have been known to be tyrosine hydroxylase (TH) immunoreactive for some time (Dubach *et al.*, 1987; Tashiro *et al.*, 1989a; Tashiro *et al.*, 1989b; Mao *et al.*, 2001), detailed knowledge of their electrophysiology has emerged only recently (Ibanez-Sandoval *et al.*, 2010). These neurons are immunopositive for glutamic acid decarboxylase $(GAD)_{65}$ and GAD_{67}, necessary for synthesis of GABA (Betarbet *et al.*, 1997; Cossette *et al.*, 2005; Mazloom & Smith, 2006). They further express the dopamine transporter DAT and receptors D2 and D3, with suppression of dopaminergic tone during neuronal development known to increase the number of striatal THIs (Betarbet *et al.*, 1997; Palfi *et al.*, 2002; Tande *et al.*, 2006; Busceti *et al.*, 2012). They are preferentially

expressed in matrix with a majority located within 100 µm of the patch-matrix boundary (Huot & Parent, 2007; Unal *et al.*, 2011). Tyrosine hyroxylase-positive interneurons (THIs) can broadly be divided into four subtypes, termed simply Type I-IV.

Morphologically, axonal projections from these neurons are highly branched with branches beginning very proximally to the soma, thus no primary axon is obvious. Type I neurons exhibit medium sized, polygonal somata. Types II-IV are similarly sized with round somata and are not easily distinguishable from one another. Type I neurons receive cortical inputs with all THIs receiving glutamatergic and GABAergic innervations revealed during intrastriatal stimulation (Ibanez-Sandoval *et al.*, 2010). Types I and II receive GABAergic input from MSNs with paired recordings having shown that single APs in MSNs are sufficient to delay firing in these neurons. Types I-III form GABAergic synapses with MSNs (Ibanez-Sandoval *et al.*, 2010) and have been shown to be involved in the cholinergic interneuron burst-pause response effects upon MSNs (Luo *et al.*, 2013).

The electrophysiological properties of THIs are shown in Figure 1.8 with each subtype expressing discrete properties. Whilst some characteristics may be similar to “classical” interneurons, no THIs express PV, SSN, NOS or NPY. Below is a summary of each subtype’s properties from Ibanez-Sandoval *et al.* (2010).

Type I comprise 60% of THIs and are characterised by very high input resistances (up to 1.5 GΩ), a resting Vm ~-70 mV and long AP duration of up to 1.9 ms. These neurons also display marked spike accommodation with an inability to maintain firing during moderate current injection. A subset exhibit long lasting plateau potentials with strong depolarisation that is dependent upon L-type Ca^{2+} channels. Around 12% are spontaneously active with a firing frequency of 4-11 Hz *in vitro*. AP duration at half amplitude is only 0.78 ms on average. Upon hyperpolarisation, a majority produce a sag that is dependent upon I_h with rebound spiking after cessation of hyperpolarising current injection in some neurons.

Type II neurons comprise 13% of THIs with moderate input resistances (average 429 MΩ) and resting Vm of -77 mV. They generate continuous APs with short duration at half amplitude (0.3-0.53 ms) with firing rates up to 250 Hz. Spikes are

followed by large AHPs of 16-25 mV. Like Type I, these neurons also present a sag when subject to hyperpolarising current injections.

Only 6% of THIs are Type III neurons. Their resting Vm of -89 mV is the lowest of all THIs and a low input resistance of 150-205 MΩ. They display strong inward rectification below -80 mV. At high current input they are incapable of maintaining spiking but can maintain firing under moderate current injection. Like Type I neurons, they exhibit L-type Ca^{2+} channel-dependent plateaus.

Type IV neurons comprise 21% of THIs. Their properties resemble LTSIs in their high input resistances and HCN-dependent sag response to hyperpolarizing current that generates rebound spikes upon recovery. However their lack of plateau potentials and shorter AP durations (0.4-0.85 ms) distinguish them from LTSIs.

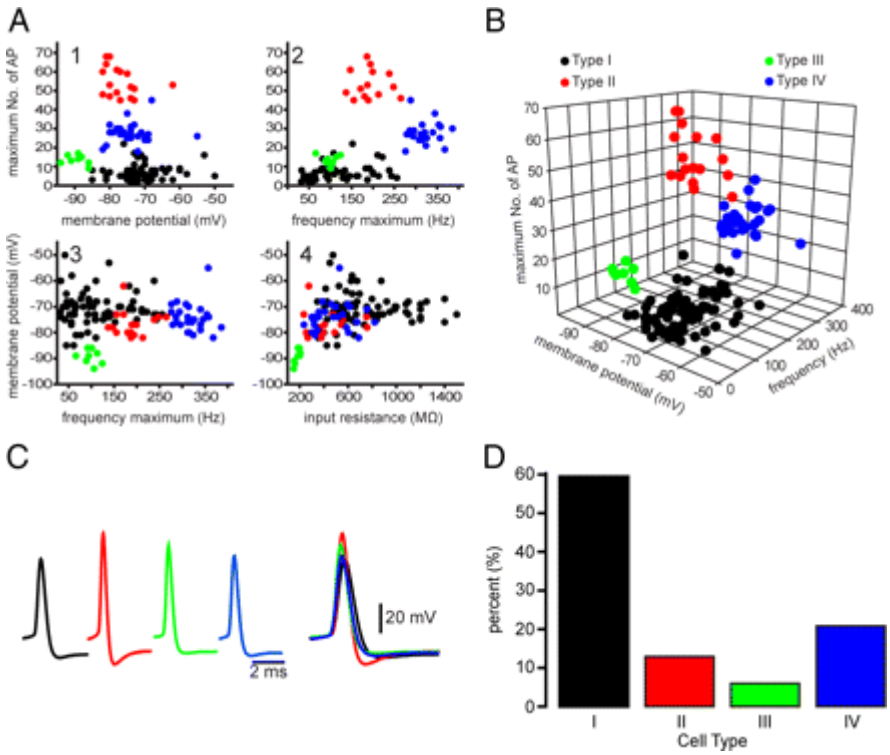

Figure 1.8: Electrophysioogical characteristics of tyrosine hydroxylase-positive interneurons. Four distinct subtypes exist, referred to as Type I-IV. **(A)** Two-dimensional scatter plots of various electrophysiological parameters. **(B)** 3-D scatter plot showing 4 distinct clusters of electrophysiological characterisitics. **(C)** Averaged action potentials from cell Types I–IV **(D)** Histogram of cell types as a percentage of all THIs. Adapted from Ibanez-Sandanoval *et al.* (2010).

1.9.7 Calretinin-positive interneuron

Little information is available concerning calretinin-positive interneurons (CRIs), however they are known to be GABAergic (Kubota *et al.*, 1993). These neurons comprise 0.5% of striatal neurons in rats (Rymar *et al.*, 2004). In primates, a greater proportion of neurons are CRIs and these are preferentially located in the caudate nucleus (Wu & Parent, 2000). At least three morphological subtypes of CRIs exist of varying somatic size and dendritic properties (Schlosser *et al.*, 1999; Wu & Parent, 2000; Rymar *et al.*, 2004; Tepper *et al.*, 2010) however their respective electrophysiological properties have yet to be explored. Their afferent and efferent connections are also elusive, however it is known they do not receive centromedian thalamic input and only 10% exhibit c-fos expression (a marker for recent neuronal firing) from cortical excitation (Parthasarathy & Graybiel, 1997; Sidibe & Smith, 1999).

1.10 Aims

The primary aim of this project is to investigate the nature of physiological activation of presynaptic $GABA_B$ receptors on corticostriatal terminals. At both cortical and thalamic inputs, presynaptic $GABA_B$ receptors are expressed both at the edge of the active zone and at extrasynaptic sites (Lacey *et al.*, 2005).

Whilst these receptors have been shown to be activated by exogenous application of agonists (Calabresi *et al.*, 1990, 1991; Nisenbaum *et al.*, 1992, 1993; Barral *et al.*, 2000), there have been no studies regarding the source of endogenous GABA release. Therefore, information regarding whether these receptors are activated physiologically does not currently exist.

MSNs are an obvious candidate for this role as they form lateral GABAergic connections with neighbouring MSNs of both the same and of neighbouring pathways (Bouyer *et al.*, 1984; Bolam *et al.*, 1988; Yung *et al.*, 1996) which have already been shown to modulate cortical input through other neurotransmitters (Blomeley *et al.*, 2009; Blomeley & Bracci, 2011).

The characteristics of this modulation, such as its effect, magnitude, time course and connection probability are of interest as connections of this type have been postulated as important contributors to action selection (Bar-Gad *et al.*, 2003; Wickens *et al.*, 2007). More generally, this would help to understand models of BG function and disease.

Modulation in the manner described was tested by evoking endogenous release of GABA by antidromic stimulation of MSN projection axons in the globus pallidus as previously demonstrated (Guzman *et al.*, 2003; Blomeley *et al.*, 2009; Blomeley & Bracci, 2011). Using this method, a group of MSNs can be selectively activated and endogenous GABA released. These stimulations occurred in conjunction with a single MSN having undergone the whole cell patch clamp technique and preceded cortical stimulation. This experimental protocol allowed cortical stimulation on a single MSN to be recorded and the effect of endogenous neurotransmitter release to be assessed. Using this method, we sought to test the hypothesis that endogenous release of GABA from lateral MSN-MSN connections can suppress glutamate release from cortical terminals onto MSNs by activation of presynaptic $GABA_B$ receptors.

We further sought to quantify the contribution, if any, of the action upon these receptors by other striatal GABAergic sources, i.e. the GABAergic interneurons. This was undertaken through paired whole-cell patch experiments of both MSN-MSN and MSN-interneuron pairs. By evoking action potentials in the second patched neuron, we were able to measure its effect upon stimulated glutamatergic responses in a patched MSN. Previous reports have used these techniques to show presynaptic inhibition at corticostriatal synapses by ACh, opioids and substance P (Pakhotin & Bracci, 2007; Blomeley & Bracci, 2008; Blomeley *et al.*, 2009; Blomeley & Bracci, 2011). Figure 1.9 details the primary circuits of investigation.

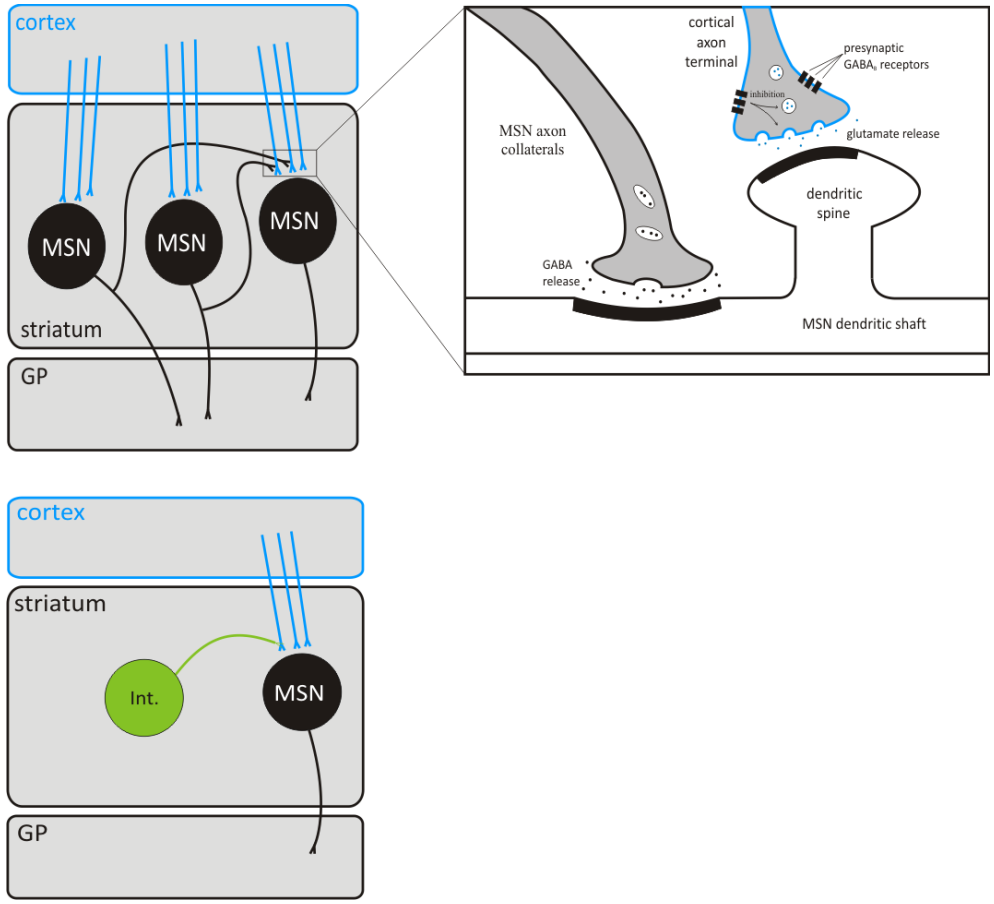

Figure 1.9: Principal circuits of investigation. Top: projections of cortical and MSN axons. Right: Location of synapses of glutamatergic and GABAergic synapses at the MSN dendrite. Bottom: projections of cortical, MSN and interneuron axons. Int; interneuron.

Chapter Two: Materials and methods

Experiments were conducted on male and female P14-21 Sprague-Dawley rats and P14-21 BAC transgenic mice heterozygous for the attachment of the NPY promoter to humanized Renilla green fluorescent protein (GFP) (BAC-npy; stock 006417, Jackson Laboratory, Bar Harbor, ME, USA). These mice express GFP in neurons where NPY is also expressed. Thus in the striatum of these mice, only LTSIs and NGFIs express NPY (Partridge *et al.*, 2009; Ibanez-Sandoval *et al.*, 2011). Rats and mice underwent cervical dislocation in accordance with the UK Animals (Scientific Procedures) Act 1986. Following rapid brain removal, parasagittal slices (200-250 μm thick) were cut using a vibroslicer (Campden instruments) in a cutting solution of artificial cerebro-spinal fluid (ACSF; mM concentrations: 124 NaCl, 2 KCl, 1 $MgSO_4$, 1.25 KH_2PO_4, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose) containing 1mM kynurenic acid, maintained at 25°C and oxygenated with 95% O_2, 5% CO_2 gas. As a non-specific glutamate antagonist, we added kynurenic acid to the cutting medium to reduce neuron excitotoxicity. Kynurenic acid also readily washes from glutamate receptors and thus would not affect subsequent experiments. After cutting, slices were maintained for at least 30 minutes in ACSF without kynurenic acid. During recording, slices were superfused with ACSF (1.5-2 mL/min) and visualised using 10x and 40x water-immersion objectives using infrared and differential interference contrast microscopy.

GFP was visualised through epifluorescent excitation with a mercury lamp (Olympus U-RFL-T) in conjunction with standard GFP filters. Initially, LTSI or NGFI candidates were identified by their differing fluorescence intensities as discerned by visual assessment and comparison to other fluorescent neurons at similar slice depth. Following visual identification, the neuron type was confirmed after patching by their distinctive electrophysiological properties (Ibanez-Sandoval *et al.*, 2011). Generally, we found that NGFIs fluoresced at somewhat greater intensities than LTSIs and subjective visual judgement of these intensities correctly identified the intended neuron type in ~75% of cases. MSNs and FSIs were also identified based on their electrophysiological features (Wilson & Groves, 1981; Kawaguchi, 1993; Ibanez-Sandoval *et al.*, 2010).

Whole-cell recordings of striatal neurons were conducted using glass pipettes filled with intracellular solution (in mM: 120 K-gluconate, 20 KCl, 0.04 EGTA, 12 HEPES, 2 $MgCl_2$, 4 Na_2ATP, and 0.4 NaGTP, adjusted to pH 7.3 with KOH). Pipette resistance was 3-7 MΩ. Recordings were carried out in current-clamp configuration using bridge amplifiers (BA-1S, BA-01X; NPI, Germany) connected to a micro1401

analog-digital converted (5 kHz sampling) driven by Signal software. Input resistance was monitored with small negative current injections.

Single patch experiments testing $GABA_B$ effects were conducted in the continuous presence of antagonists of the following receptors: $GABA_A$ (100 µM picrotoxin), D1 (10 µM SCH 23390 hydrochloride), D2 (3 µM S-(-)-Sulpiride), NK1 (10 µM L-732,138), µ, κ and δ opioid (10 µM naloxone hydrochloride), nicotinic (100 nM nicotine or 10 µM tubocurarine chloride) and muscarinic (25 µM atropine sulphate). To confirm the effects of $GABA_B$, we subsequently added the $GABA_B$ antagonist CGP 52432 (1 µM). Drugs were added at a rate of 1.5-2.5 mL/min. Data acquired in the first 10 minutes after drug application were not considered in analysis in order to allow diffusion of drugs to intended concentration.

Paired recording experiments involving two MSNs were conducted in the presence of $GABA_A$, opioid and NK1 receptor antagonists. Paired recording experiments from NGFI-MSN and MSN-LTSI pairs were conducted in the presence of $GABA_A$ receptor antagonists only. Experiments testing the effects of NPY and NO were tested in the presence of $GABA_A$ and $GABA_B$ receptor antagonists. We found that at times, paired patching could be problematic as after patching of the first neuron, moving the second pipette tip into the tissue would distort the tissue and disrupt the first patch. Patching pairs of neurons that are further apart is undesireable as it may reduce the probability of direct interactions. Therefore, in some cases we placed both tips into the tissue near the target neurons before start of patching of either neuron to reduce subsequent tissue distortion. In either case, great care was taken to ensure that patchng of the second neuron did not damage the patch of the first neuron. If such damage occurred, the first neuron was discarded.

In all experiments, it was important to ensure that neurons were healthy for the duration of the experiment. We only used neurons where neuron properties were clear and stable; where resting Vm was constant and stable; APs were stable and reproducible; and noise was low (as this may be an indication of poor or incomplete patch). In most experiments, we tested input resistance throughout. Where input resistance altered by more than 25% (not due to drug application), we discarded experiments.

In all cases, testing of neuron electrophysiological properties was conducted in the absence of drugs. Testing the presence of direct connections between NGFI-MSN pairs was also conducted in the absence of drugs.

Glutamatergic excitatory post synaptic potentials (EPSPs) were evoked by electrical stimulation of the corpus callosum (CC), as in previous studies (Blomeley *et al.*, 2009; Blomeley & Bracci, 2011). EPSPs were recorded in all experiments from MSNs located in the dorsomedial striatum. In most experiments, a single CC stimulus was delivered continuously every 10 s. CC stimulation intensity was adjusted to produce EPSPs of 5-15 mV amplitude.

In single recording experiments, two protocols were used. The first featured simple repetitions of single CC stimuli every 10 s, with responses recorded in MSNs (Figures 3.2, 3.3, 4.4) every other CC stimulus was preceded by a train of stimuli (5 stims, 50 Hz) delivered by a second stimulator placed in the globus pallidus (GP) to activate antidromically MSN axons (fig 3.4 A, B), as previously described (Blomeley *et al.*, 2009; Blomeley & Bracci, 2011). GP stimulation intensity was adjusted so that no antidromic spike was observed in the recorded MSN (Blomeley & Bracci, 2009) and to ensure that any antidromic-orthodromic depolarisation had returned to baseline before CC stimulation. In paired recording experiments, every other CC stimulus was preceded by a train of action potentials (5 spikes at 50 Hz) elicited by short (5 ms) current injections in a second nearby patched neuron (Figures 4.2, 4.3) as in previous studies (Blomeley *et al.*, 2009; Blomeley & Bracci, 2011). These trains of stimuli/spikes preceded the CC stimulus by 0.5-3.0 s (the preceding intervals were always calculated from the first stimulus/spike of the train to the time of the CC stimulation). Both single and paired experiments would cycle continuously between protocol(s) that featured prestimulation and the protocol in the absence of prestimulation for the duration of the experiment. CC and GP stimuli were in the range of 10-300 µA. We found that at the lower end of this stimulation range (<100 µA) it was often difficult to evoke EPSPs of amplitude of more than 2 or 3 mV except in a narrow area of the CC. However we found that after patching, it was usually still possible to move the CC stim without disrupting existing whole cell-patches and thus most experiments herein feature CC stimulation amplitude in the 30-100 µA range.

In some experiments, input resistance was periodically tested using negative current injections of 0.2-0.5 s duration and 10-50 pA step current intensity to test

for postsynaptic effects, steps began 0.2 s after start of GP stimulation trains/invoked AP train in cells in order to test these effects upon input resistance. These steps were then compared to similar steps during control conditions. Experiments were only considered for step analysis where Vm of patched cell returned to baseline following prestimulation and where significant inhibition of cortical inputs was seen. In experiments where paired-pulse ratio (PPR) was tested, PPR was calculated as 2nd EPSP/1st EPSP.

Where these steps were used to test NGF and LTSI Ih ratios, Ih ratio was taken as

$$Ih = \frac{baseline - (mean\ (t100 - t200))}{baseline - (mean\ (t400 - t500))}$$

where *t*x is x milliseconds after start of a 500 ms current step.

Spike AHPs were measured as the difference between spike threshold (where change in membrane potential exceeded 10 mV/ms) and the lowest point following the spike.

When testing single neurons only, an analysis of variance (ANOVA) test for multiple groups followed by post-hoc t-tests with Bonferroni correction was used to test for significant differences between responses in several conditions (e.g. control vs 0.5 s prestim or 0.5 s prestim vs 1 s prestim).

When testing across a group of neurons, mean responses for each condition in a single neuron were calculated and then normalized against the control mean for that neuron to produce a figure for inhibition in each state. A paired Student's t-test was then used to test across all neurons (e.g. % inhibition in absence of CGP 52432 vs % inhibition in presence of CGP 52432).

In all tests, the significance threshold was set as $p<0.05$ however where lower p-values have been found from tests, this has been described in the results (eg. $p<0.01$ or $p<0.001$).

Chapter Three: Medium spiny neurons presynaptically inhibit corticostriatal transmission through $GABA_B$ receptors

3.1 Introduction and aims

How the striatum processes patterns of cortical input is important in understanding theories of action selection.Striatal MSNs receive most of the excitatory cortical input into the striatum and are the striatal principal neuron (Wilson, 2004). Thus understanding connections between cortical axon terminals and striatal MSNs is particularly important in understanding BG function (Bar-Gad *et al.*, 2003; Wilson, 2007). $GABA_B$ receptors have been shown to be expressed both pre- and post-synaptically at the corticostriatal synapse (Lacey *et al.*, 2005) and it has been shown previously that application of a $GABA_B$ agonist can inhibit the release of glutamate at this synapse (Calabresi *et al.*, 1990, 1991; Nisenbaum *et al.*, 1992, 1993; Barral *et al.*, 2000). However the physiological source, if any, of activation of these receptors has not been shown.

Therefore, we wished to investigate the source of this activation. In addition to their main axon projections outwith the striatum, MSNs also form lateral connections with other MSNs. These connections form synapses on the dendritic shaft of MSN dendrites, anatomically close to corticostriatal synapses (Wilson & Groves, 1980; Somogyi *et al.*, 1981; Kawaguchi *et al.*, 1995). Furthermore, it has previously been shown that release of co-transmitter by MSNs at MSN-MSN synapses can modulate release of glutamate from nearby cortical axon terminals (Blomeley & Bracci, 2008; Blomeley *et al.*, 2009; Blomeley & Bracci, 2011).

We therefore hypothesised that release of GABA from MSN-MSN axon collaterals would be capable of inhibiting glutamate release in a $GABA_B$ receptor-dependent manner and wished to characterise this phenomenon.

3.2 Results

3.2.1 Properties of striatal neurons

Our experiments required identification of striatal neuron types in rat. Morphologically, neurons of the non-cholinergic type are similarly shaped and cannot be distinguished comprehensively using IR-DIC visualisation alone. Therefore, after patching, neurons were identified using their distinctive electrophysiological properties (Wilson & Groves, 1980; DiFiglia & Aronin, 1982; Bolam *et al.*, 1984; Kawaguchi, 1993; Kawaguchi *et al.*, 1995). We tested the properties of patched neurons using positive and negative current steps. We were able to reproducibly identify neurons of the most commonly studied subtypes (Figure 3.1). Of 15 analysed MSNs, we identified properties commonly found in these neurons. These included low resting membrane potential of -78.6±0.9 mV, low input resistance of 183±14MΩ and AHPs of 12.7±0.7 mV. We also saw common features including slow depolarisation to first action potential and marked inward rectification (Wilson & Groves, 1981; Kita *et al.*, 1984; Wilson & Kawaguchi, 1996).

We also identified neurons that could be classified as FSIs. These neurons exhibited all the key features of FSIs including very high maximum firing rate, short duration AHPs and bursts of spikes interspersed with subthreshold membrane oscillations in response to constant current injection (Figure 3.1). From 12 neurons (average animal age 18.1 d), we observed average resting membrane potentials of -70.2±2.4 mV, IRs of 184±17 MΩ and AHP amplitudes of 14.3±1.6 mV. AHP amplitudes and resting membrane potentials correlated well with animals at a similar developmental age (Chesselet *et al.*, 2007). Input resistances were higher in our observed neurons than in previous described animals (Kawaguchi, 1993; Chesselet *et al.*, 2007). However we feel that the extant properties observed allow us to positively identify these neurons as the fast-spiking type.

From neurons that were of the non-LAI morphology (LAIs are conspicuous due to their greater size), we also identified LTSIs. All patched LTSIs exhibited rebound depolarisations upon cessation of a negative current step, high input resistances and either depolarised resting membrane potentials or spontaneous firing. From 5

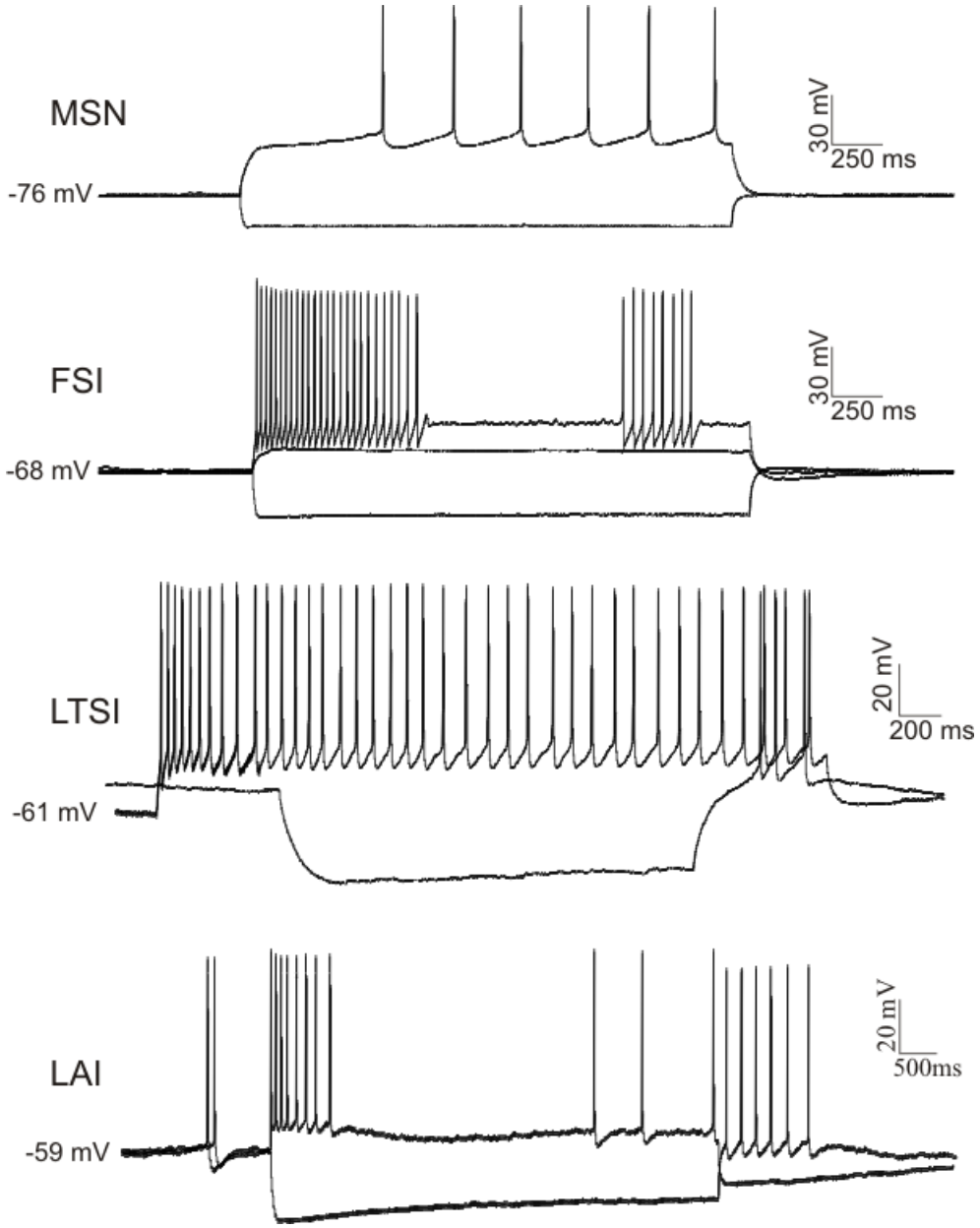

Figure 3.1: Responses of striatal neurons to current steps. Electrophysiological characteristics. Patched neurons exhibited discrete properties that allowed easy identification.

neurons (average animal age 17.0 d) we observed average IRs of 607±61 MΩ and AHPs of 15.4±0.4 mV. 3/5 neurons were spontaneously active. 2/5 fired in an irregular spontaneous fashion, alternating spontaneously between periods of quiescence and regular firing. During periods of quiescence, resting membrane potential in these 2 neurons was -52.7±1.5 mV. These properties are consistent with those previously described for LTSIs in rats (Kawaguchi, 1993; Koos & Tepper, 1999).

Many of our experiments required a large number of repetitions of each protocol in an individual neuron/experiment in order to achieve statistically significant effects due to the high variability of corticostratal responses (Figure 3.2). Experiments often required in excess of 100 frames to achieve significance. Across 27 experiments where we measured responses to cortical stimulation in control solution, mean CV was 0.23±0.05 (range 0.09-0.41).

3.2.2 Application of a $GABA_B$ agonist presynaptically inhibits corticostriatal transmission

$GABA_B$ receptors are found presynaptically at corticostriatal synapses and are known to suppress glutamate release at these synapses (Calabresi *et al.*, 1990, 1991; Nisenbaum *et al.*, 1992, 1993). We attempted to confirm these results in our own experiments. In the presence of picrotoxin, a specific $GABA_A$ antagonist, we stimulated in the CC and measured glutamatergic responses in MSNs that had undergone the whole-cell patch-clamp technique (Figure 3.3 A, B). We then compared these to responses after bath application of a $GABA_B$ agonist, baclofen. Baclofen reduced MSN responses to cortical stimulation in a concentration dependent manner (Figure 3.3 C, D, E). Over 3 experiments, bath application of 0.25 µM baclofen significantly reduced MSN responses by 42.3±7.7% (paired t-test, $p<0.01$). We also used a paired pulse protocol to test if these effects were presynaptic, PPRs increased from 0.85±0.02 in control conditions to 1.17±0.07 in 0.25 µM baclofen ($p<0.05$; Figure 3.3 D). In all experiments, increases in baclofen concentration led to an increase in PPR. From these results we concluded that the $GABA_B$-dependent inhibition had a presynpatic locus of effect.

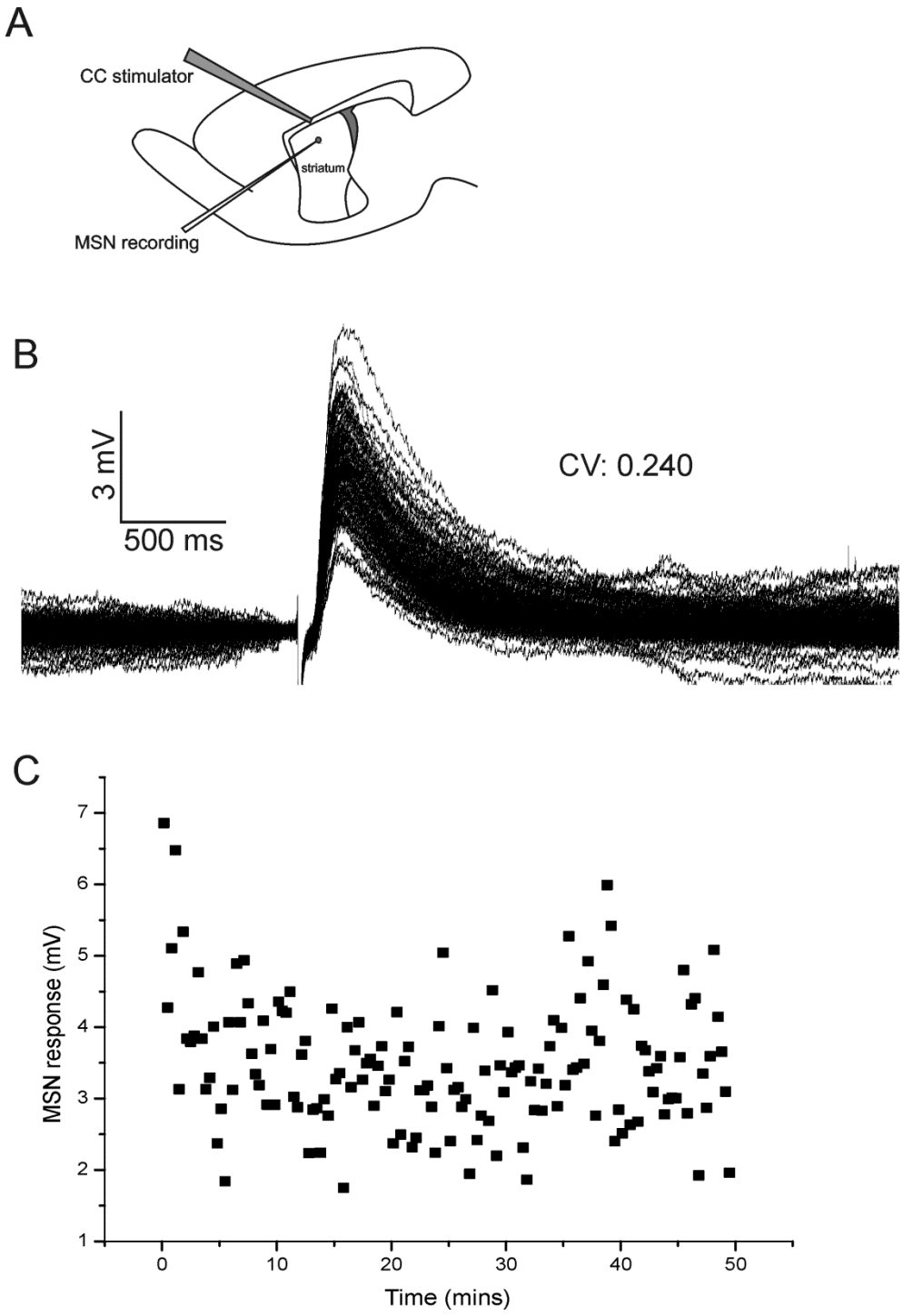

Figure 3.2: Corticostriatal responses display marked variability. (A) Experimental design. MSNs were patched in the dorsolateral striatum and simultaneously, CC was stimulated by a separate stimulator. Glutamatergic responses were measured in MSNs. **(B)** In this representative example, 137 EPSPs from a single neuron were superimposed. **(C)** A scatterplot over time of responses shown in (B).

3.2.3 Antidromic stimulation of MSNs inhibits cortical inputs onto MSNs

Electrical stimulation of the GP triggers antidromic spikes in both striatonigral and striatopallidal MSNs since their axons pass through or terminate in this region (Lopez-Huerta *et al.*, 2013). These antidromic spikes then trigger orthodromic spikes in MSN axon collaterals, causing neurotransmitter release (Guzman *et al.*, 2003; Lopez-Huerta *et al.*, 2008; Blomeley *et al.*, 2009; Blomeley & Bracci, 2011; Lopez-Huerta *et al.*, 2013). We therefore stimulated the GP to evoke GABA release from MSNs and to test its effect on glutamate responses evoked by CC stimulation (Figure 3.4 A, B). These experiments were carried out in the presence of antagonists for $GABA_A$, dopamine, opiate, NK1 and acetylcholine receptors (see Methods) to prevent unwanted activation of these receptors by GP stimulation and MSN firing.

In juvenile rats, GP stimulation (preceding CC stimulation by 500 ms) significantly reduced the amplitude of responses to subsequent CC stimulations across 21 neurons (paired t-test, $p<0.001$). Testing of inhibition in individual neurons (ANOVA with post-hoc Bonferroni; $p<0.05$) showed significant inhibition in 12/21 neurons, average inhibition 6.7±1.1% (Figure 3.4 C,D, Figure 3.5 A). Average inhibition in significant neurons was 10.0±0.8%. In order to test if these effects depended on $GABA_B$ receptors, in 19 of these experiments (in 11 of which significant inhibitory effects were observed in control solution) we subsequently applied the specific antagonist CGP 52432 (1 µM), while continuing to apply the same stimulation protocol. In all cases in the presence of CGP 52432, GP stimulation did not cause significant inhibition of CC-evoked responses. The effects observed in individual experiments in the absence and in the presence of CGP 52432 are illustrated in the histograms of Figure 3.4 C and in the trend plots of Figure 3.4 E. In previous studies, $GABA_B$ receptor agonists did not cause detectable postsynaptic effects in MSNs, and paired-pulse stimulation experiments pointed to a presynaptic site of action (Calabresi et al., 1992; Nisenbaum et al., 1993). Our data (Figure 3.3) support this. Whilst our experiments with baclofen featured a paired pulse protocol, such a protocol could not be used in our GP stimulation experiment. This is because the concentration of the $GABA_B$ agonist (in this case the endogenously released GABA) is not constant as the GABA concentration would decrease over time following antidromically-evoked APs. Therefore it would not be certain whether changes in PPR were due to locus of GABA effect or GABA concentration at the synapse.

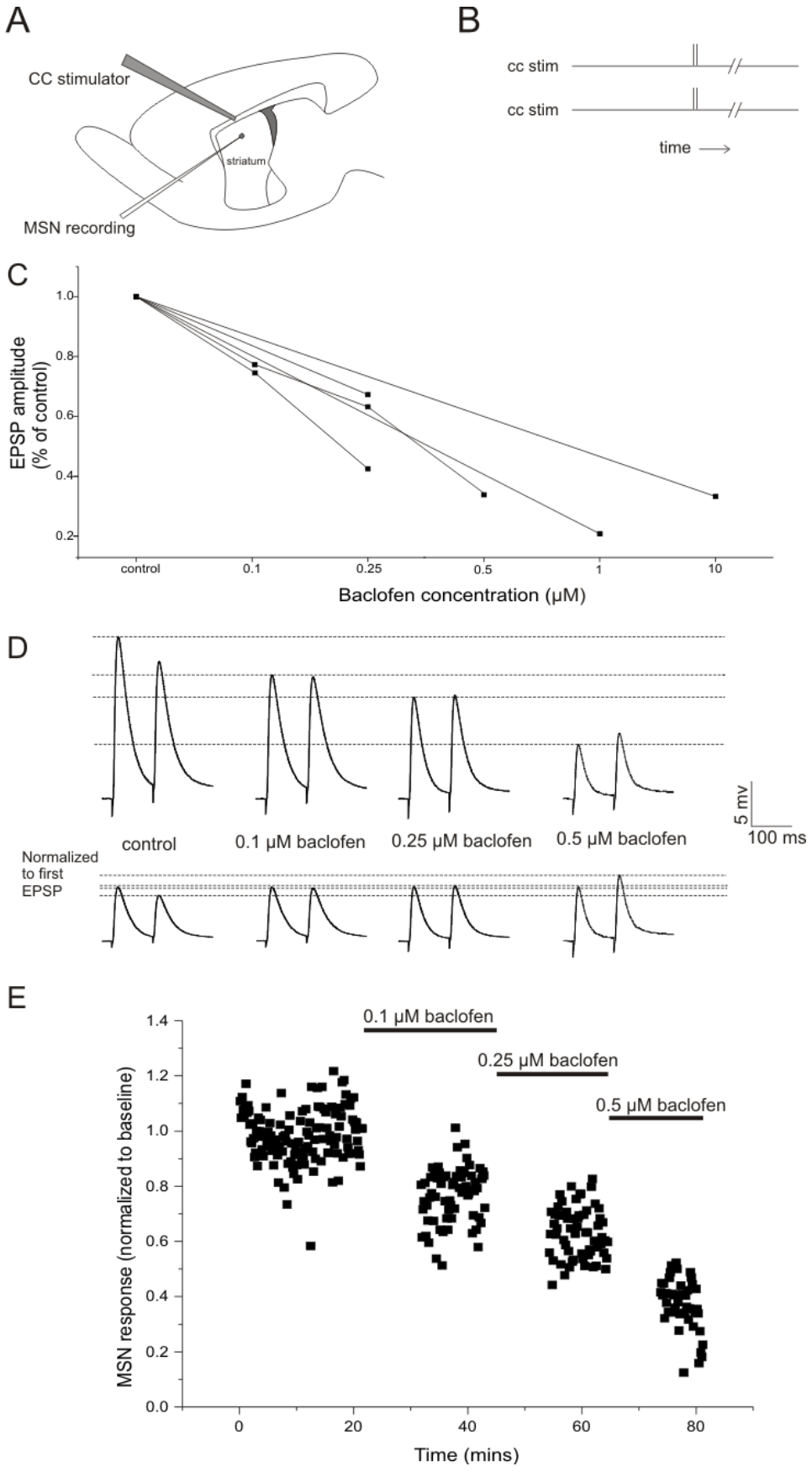
A
CC stimulator
striatum
MSN recording
B
cc stim
cc stim
time
C
EPSP amplitude (% of control)
1.0
0.8
0.6
0.4
0.2
control
0.1
0.25
0.5
1
10
Baclofen concentration (μM)
D
control
0.1 μM baclofen
0.25 μM baclofen
0.5 μM baclofen
5 mv
100 ms
Normalized to first EPSP
E
0.1 μM baclofen
0.25 μM baclofen
0.5 μM baclofen
MSN response (normalized to baseline)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Time (mins)

Figure 3.3: Baclofen presynaptically suppresses corticostriatal glutamate release. (A) Positioning of recording and stimulating electrodes. An MSN was recorded in the dorsolateral striatum. Corticostriatal fibres were activated by stimulation in the CC. **(B)** Stimulation sequence. A simple protocol in which a pair of stimuli in the CC evoked responses in a patched MSN. Stimulation pairs were 100 ms apart. This protocol was repeated every 10 s. After control responses were recorded, baclofen was added for comparison, sometimes in stages at increasing concentrations. **(C)** Line plot of baclofen effects. Each line shows multiple concentrations tested in a single neuron. Some experiments feature only a single drug concentration, others feature 2 or 3. Baclofen reduces MSN responses in a concentration-dependent manner. **(D)** Averaged traces from an experiment featuring 3 concentrations of baclofen. Top: unmodified traces show first EPSP is reduced by baclofen in a concentration-dependent manner. Bottom: traces with normalized first spikes show a concentration-dependent increase in paired pulse ratio. From left to right, traces are an average of 64, 68, 62 and 20 repetitions respectively. **(E)** Normalized scatterplot of first EPSP of experiment shown in (D),

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In order to test for possible postsynaptic contributions to the effects caused by $GABA_B$ receptor activation, in some experiments, we applied a 200 ms negative current step (10-40 pA) 200 ms after GP stimulation. The membrane potential displacement caused by these steps was measured at the end of the current injection, to minimise the effects of any residual depolarisations induced by the GP stimuli. As illustrated by the example of Figure 3.4 F, in 5/5 experiments (in which significant $GABA_B$ receptor-mediated effects on CC-evoked responses were present), the input resistance was not significantly different when the step was preceded by GP stimuli (paired t-test, average IR in CGP: 100.8±2.0% of control).

We concluded that synchronous activation of MSNs caused inhibition of glutamatergic synapses onto MSNs, through GABA release leading to the activation of presynaptic $GABA_B$ receptors with no detectable postsynaptic effect.

3.2.4 Presynaptic inhibition is maximal at 500 ms intervals and disappears at 3 s

In order to determine the time course of the inhibition of glutamatergic responses caused by antidromic activation of MSNs, we carried out experiments as in Figure 3.4 but at longer time intervals between GP and CC stimulation of 1, 2 or 3 s. Due to the fact that repeated GP stimulation elicited long-tailed glutamatergic responses in MSNs, it was not technically possible to test intervals smaller than 500 ms (Blomeley *et al.*, 2009; Blomeley & Bracci, 2011). Testing of individual neurons with ANOVAs followed by post-hoc Bonfferoni t-tests showed significant ($p<0.05$)

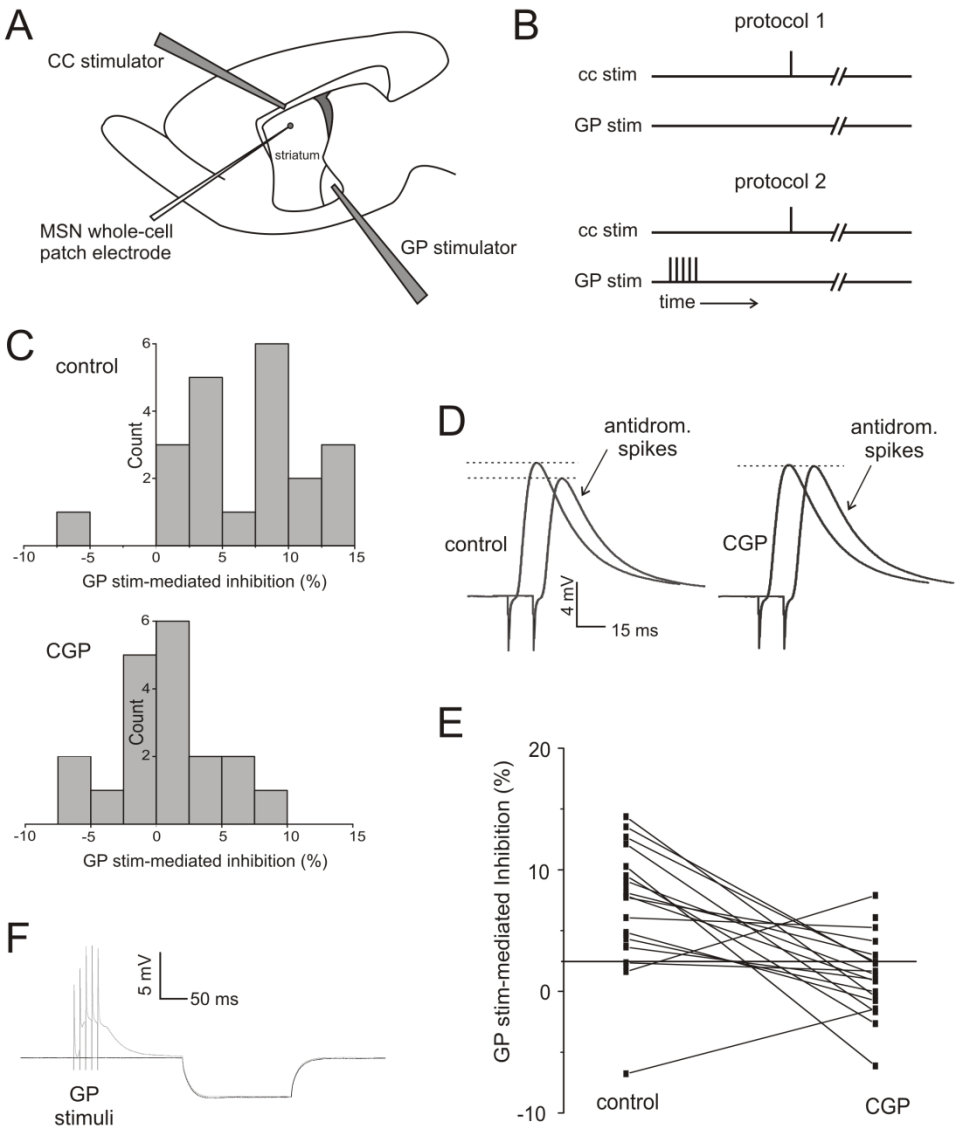

Figure 3.4: Antidromic activation of MSNs causes $GABA_B$ receptor-dependent inhibition of corticostriatal inputs. (A) Typical positioning of recording and stimulating electrodes. An MSN was recorded in the dorsolateral striatum. Corticostriatal fibres were activated by electrical stimulation delivered to the CC. MSN axons were antidromically activated by electrical stimulation delivered to the GP. **(B)** Stimulation sequence. Two consecutive stimulation protocols (each lasting 10 s) were applied repeatedly and alternately. In the first protocol, a single stimulation to the CC evoked control responses in the MSN. In the second protocol the CC stimulation was preceded (by 0.5 s) by a train of 5 stimuli in the GP, to elicit GABA release from a population of MSNs. This two-protocol cycle was applied without interruptions at least 75 times for each pharmacological condition. **(C)** Distribution of the average inhibitory effects of MSN antidromic activation on cortically evoked EPSPs observed in 21 experiments in control solution (top) and in the presence of CGP 52432 (bottom). **(D)** $GABA_B$ receptor-mediated inhibition in a representative experiment. Traces are averages of CC-evoked responses without or with preceding GP stimulation. In control solution, cortical inputs are inhibited by preceding

antidromic activation of MSNs. In the presence of CGP 52432, these inhibitory effects are abolished. **(E)** Inhibitory effects of antidromic MSN stimulation on cortical responses in individual experiments. Data as in (C). In each case, CGP 52432 was applied after the stimulation protocols had been carried out in control solution. **(F)** A representative example of the lack of effects of GP stimuli on MSN input resistance. The trace in black is the average of 90 consecutive steps (200 ms, -30 pA) applied without preceding GP stimuli. The trace in grey is the average of 90 consecutive steps (200 ms, -30 pA) applied 200 ms after the GP stimuli. Current steps were delivered every 10 s and GP stimuli preceded every other step.

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inhibition in 5/13 neurons at 1 s intervals (average across all neurons 4.0±1.0%; Figure 3.5 A); experiments at 2 s intervals showed significant inhibition in 6/21 of cases (average across all neurons 3.8±0.6%; Figure 3.5 A). Experiments at 3 s intervals never showed inhibition in 6 experiments (average inhibition 0.5±0.5%). ANOVA testing across all neurons showed significant ($p<0.01$) differences between time intervals. Post hoc tests showed inhibition was significant ($p<0.05$) at 0.5, 1 and 2 s intervals but not 3 s. Tests did not show significant differences between 0.5 vs 1 s or 1 vs 2 s. However inhibition at 2 s was significantly greater than at 3 s ($p<0.001$).

We also conducted experiments featuring two different time intervals between CC and GP stimulations (Figure 3.5 B). These experiments were similar to those described above but consisted of three repeated cycles: 1) without GP stimulation 2) GP stimulation preceded CC stimulation by 500 ms, and 3) GP stimulation preceded CC stimulation by either 1, 2 or 3 s. In all experiments, the inhibitory effects were larger for 500 ms intervals than for other intervals tested. Testing of significance in individual neurons (ANOVA with postnhoc Bonferroni; $p<0.05$), in 10/14 of these experiments, significant inhibitory effects were observed at 500 ms intervals; at 1 s intervals, significant inhibition was observed in 3/4 experiments. At 2 s intervals, significant inhibition was observed in 2/4 experiments. At 3 s intervals, no significant inhibition was observed in 6 experiments. Paired t-tests of inhibition between 0.5 s and other time intervals showed that 0.5 s intervals produced significantly greter inhibition than at 1 s ($p<0.05$), 2 s ($p<0.01$) and 3 s ($p<0.001$) intervals.

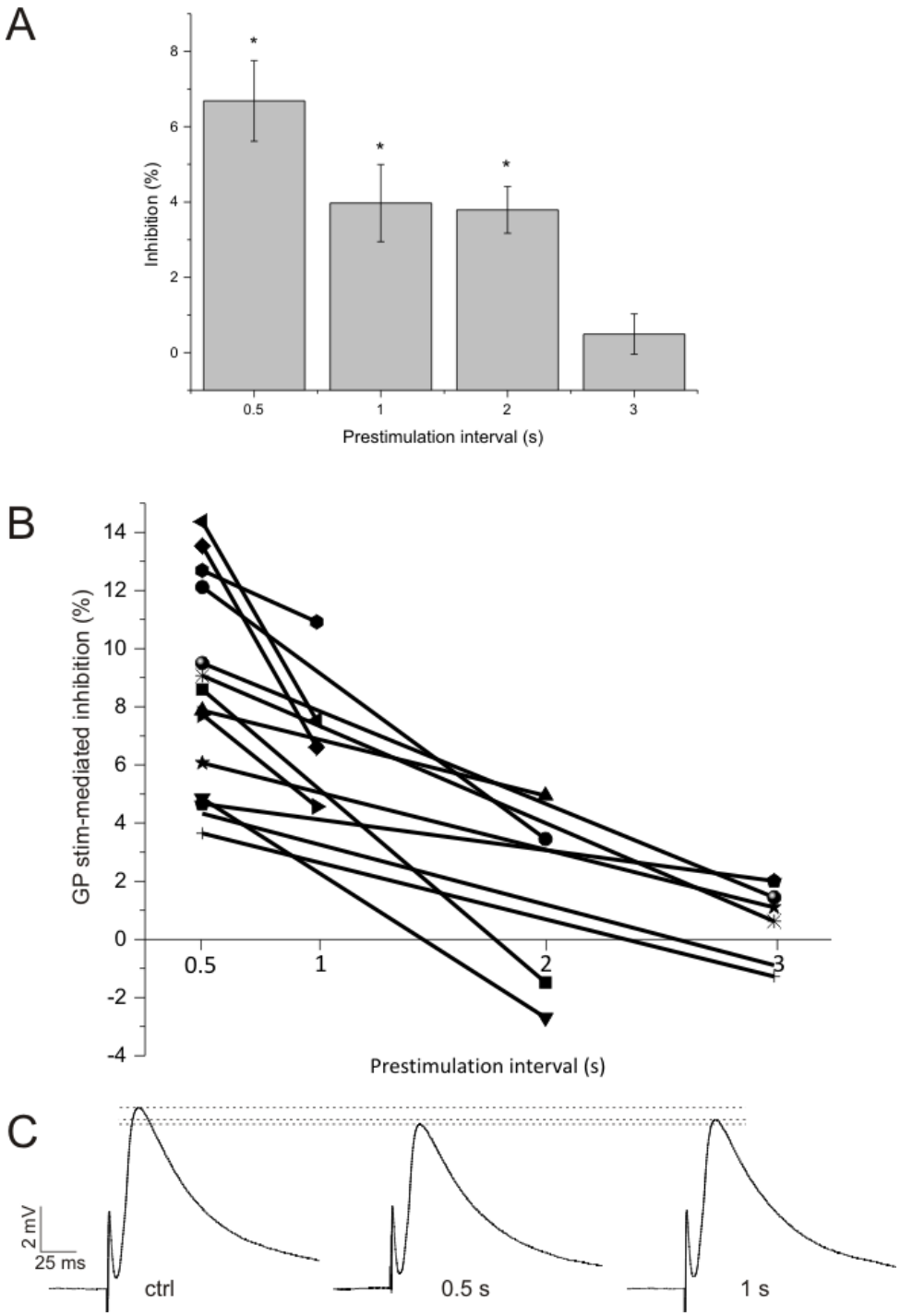

Figure 3.5: The inhibitory effects of MSN antidromic activation are maximal at 0.5 s interval. (A) Average inhibition across all tested neurons. Experiments were conducted as in Figure 3.4 but with prestimulation interval of 1, 2 or 3 seconds. Intervals of 1 or 2 s were similar to each other but exhibited less inhibition than at 0.5 s interval. 3 s intervals never showed significant inhibition. **(B)** Each line represents a single experiment. In each of these experiments, a second interval was tested in addition to 0.5 s. Thus, three consecutive stimulation protocols (no GP stim; GP stim at 0.5 s interval; GP stim at 1, 2 or 3 s interval) were applied. All experiments produced greatest inhibition at 0.5 s with declining inhibition for intervals up to 2 s. No significant inhibition was seen at 3 s intervals. **(C)** Averaged traces from a representative experiment. Significant inhibition is seen at 0.5 s and 1 s intervals; inhibition is significantly ($p<0.05$) smaller at 1 s.

Paired patch MSN-MSN experiments were also conducted to test if evoked action potentials in a single MSN were sufficient to significantly reduce cortical glutamate release (experiments conducted by V.Bagetta; experimental protocol as in Figure 4.2). However in 57/57 experiments, no significant effect was seen (Logie *et al.*, 2013).

3.3 Discussion

The main finding of this chapter is that endogenous GABA, released by a population of striatal MSNs, can depress glutamatergic inputs to MSNs by activating $GABA_B$ receptors. However, individual MSNs are unable to produce a similar effect. This effect is maximal at 500 ms intervals.

Application of baclofen, a $GABA_B$ agonist, significantly reduced corticostriatal transmission at these synapses (Figure 3.3). Our experiments also demonstrated a clear increase in PPR. Whilst we cannot rule out a postsynaptic effect in these experiments, a presynaptic effect was obvious. Nisenbaum *et al*. (92, 93) report inhibition of MSN responses to cortical stimulation at 0.5 µM baclofen concentration of 38.1±17.6% and 38.7±17.2%. Our results are in line with these, showing 42.3±7.7% inhibition at 0.25 µM concentration.

Paired recording experiments showed that a burst of five spikes in a single MSN was insufficient for $GABA_B$ receptor-mediated inhibition of glutamatergic inputs to a neighbouring MSN (experiments conducted by V. Bagetta; (Logie *et al.*, 2013). In contrast, such inhibition could be elicited by activating a population of MSNs with five antidromic stimuli delivered at the same frequency (Figure 3.4). This protocol excites similar numbers of striatopallidal and striatonigral MSNs, eliciting orthodromic spikes in their axon collaterals and synchronous release of GABA (Lopez-Huerta *et al.*, 2013). The most likely explanation for these results is that a relatively large amount of GABA needs to be released in order to activate presynaptic $GABA_B$ receptors located on glutamatergic afferents. MSN-MSN GABAergic synapses tend to be formed on the dendritic shafts, while corticostriatal glutamatergic inputs are mainly formed on dendritic spines (Bevan *et al.*, 1998; Boyes & Bolam, 2007). Therefore, activation of presynaptic $GABA_B$ receptors located on glutamatergic terminals requires diffusion of GABA in the extrasynaptic space, since the locus of effect is presynaptic. This only took place effectively

when a number of MSNs were synchronously activated. Synchronous activation of groups of MSNs has been shown to occur robustly in response to cortical burst stimulation (Carrillo-Reid *et al.*, 2008). GP stimulation causes antidromic activation of both striatopallidal and striatonigral MSNs, but it does not allow precise identification of the size of the stimulated MSN population. A pallidostriatal GABAergic projection has been demonstrated, but it targets selectively striatal FSIs rather than MSNs (Bevan et al., 1998) and would be blocked by the action of picrotoxin. Therefore it is unlikely to have played a role in the observed phenomenon.

Whilst it was not directly demonstrated in antidromic stimulation experiments that the effects of $GABA_B$ receptors were presynaptic, previous experiments carried out with exogenous agonists strongly suggest that this was the case. $GABA_B$ receptor agonists have been shown to reduce glutamatergic EPSPs of MSNs through presynaptic mechanisms, while no postsynaptic effects were seen (Calabresi *et al.*, 1991; Nisenbaum *et al.*, 1993; Barral *et al.*, 2000). This somewhat contradicts the fact that $GABA_B$ receptors are found postsynaptically on MSNs (Lacey et al., 2005). Consistent with the previous reports, we never observed $GABA_B$ receptor-mediated postsynaptic effects caused by spikes in MSN populations (Figure 3.4 F). These findings, along with our data from baclofen application (Figure 3.3), strongly suggest a presynaptic effect. Further studies will be required to reveal if postsynaptic $GABA_B$ receptors are functionally impaired or, alternatively, mediate effects that are not detected by standard electrophysiological techniques.

Glutamatergic responses were evoked by electrical stimulation of the portion of CC located between the cortex and the striatum. While this procedure can be expected to produce preferential activation of corticostriatal fibres, it is likely that some thalamostriatal axons were also activated. Presynaptic $GABA_B$ receptors are found on both corticostriatal and thalamostriatal terminals (Charara *et al.*, 2000; Lacey *et al.*, 2005). Further experiments will be required to determine if $GABA_B$-mediated inhibition differs in the two sets of afferents.

These data supplement previous experiments that have shown that different populations of MSNs control the glutamatergic terminals in opposite ways either through activation of presynaptic NK1 receptors by substance P, or activation of presynaptic μ-opioid receptors by enkephalin (Blomeley *et al.*, 2009; Blomeley & Bracci, 2011).

The time course of $GABA_B$ receptor-mediated inhibitory effects may be similar to that observed for the activation of μ-opioid receptors (Blomeley and Bracci, 2011). In that case, inhibition of glutamate inputs was found to peak 500 ms after a burst of spikes and to be still present, although reduced, after 1s and in some cases after 2 s. This is slower than substance P-mediated facilitation, that was found to peak after 250 ms (Blomeley *et al.*, 2009). In the present experiments, technical considerations prevented us from testing at intervals less than 500 ms (see section 5.4). This technical limit could be surmounted through optogenetic activation of a population of MSNs in place of antidromic stimulation in the GP.

An important difference between the previously studied presynaptic interactions and those mediated by $GABA_B$ receptors is that the former could be elicited by spikes in individual MSNs. In the case of GABA acting on $GABA_B$ receptors, the effects require synchronous activation of several MSNs. Nevertheless, the different time course, direction and magnitude of these modulatory interactions is likely to give rise to specific network dynamics that may be key to striatal function. Collectively, these observations provide a novel picture of the striatal network, in which rapid feed-forward and feed-back GABAergic inhibition through ionotropic $GABA_A$ receptors is accompanied by slower presynaptic metabotropic interactions mediated by peptides, GABA and acetylcholine.

Chapter Four: NPY and NPY-expressing neurons modulate corticostriatal transmission

4.1 Introduction and aims

Striatal GABAergic neurons are known to have a significant capacity to modulate the output of MSNs. In particular, both FSIs and NGFIs have a strong ability to inhibit MSNs through direct GABAergic neurotransmission which can abrogate AP generation (Koos & Tepper, 1999; Kubota & Kawaguchi, 2000; Gittis *et al.*, 2010; Ibanez-Sandoval *et al.*, 2011). Furthermore, LTSIs also form weaker GABAergic connections with MSNs (DiFiglia & Aronin, 1982; Aoki & Pickel, 1988; Kubota & Kawaguchi, 2000; Gittis *et al.*, 2010). We therefore hypothesised that these neurons could inhibit glutamate release from corticostriatal synapses by activation of $GABA_B$ receptors. We were able to selectively target NGFIs and LTSIs through use of NPY-EGFP expressing mice, which express a fluorescent marker only present in these striatal neurons (Ibanez-Sandoval *et al.*, 2011; Luo *et al.*, 2013).

Furthermore, we generally wished to characterise NGFIs. These neurons have only recently been characterised for the first time (Ibanez-Sandoval *et al.*, 2011) and we therefore tested their electrophysiological properties in our own recordings.

Finally, we wished to examine the role of two co-transmitters: NPY, which is expressed by NGFIs and LTSIs; and NO, which is released in the striatum by LTSIs only. The role of LTSIs in particular in striatal circuity has not been well characterised, although they are primarily thought to act through their co-transnmitters rather than GABA. NO has been implicated in both transient modulation of MSNs and plasticity of corticostriatal synapses. LTSI axon projections that terminate on MSN dendrites are known to contain nNOS (Calabresi *et al.*, 1999c; Sancesario *et al.*, 2000) and MSN dendrites contain downstream messengers of NO (Ariano *et al.*, 1982; Matsuoka *et al.*, 1992; Fujishige *et al.*, 1999; Ding *et al.*, 2004). Intrastriatal infusion of cGMP analogues and local administration of PDE inhibitors have each been shown to depolarise MSNs (West & Grace, 2004; Threlfell *et al.*, 2009). However the physiological effect of endogenous LTSI excitation on MSN corticostriatal responses has not been investigated.

NPY receptors and their mRNA are widely expressed in the striatum (Caberlotto *et al.*, 1997; Caberlotto *et al.*, 1998; Caberlotto *et al.*, 2000; Wolak *et al.*, 2003; Stanic

et al., 2006). LTSIs and NGFIs are the only striatal neurons that express NPY (Smith & Parent, 1986; Ibanez-Sandoval *et al.*, 2011). We therefore investigated whether NPY could modulate corticostriatal transmission.

4.2 Results

Our experiments featured paired recording experiments in BAC transgenic mice (see Materials and Methods), in which a MSN and another striatal GABAergic interneuron (located <100 µm apart in the dorsolateral striatum) were recorded simultaneously (Fig 4.2 A,B).

4.2.1 NPY-positive neurons exhibit two discrete subtypes

Neurogliaform interneurons (NGFIs) are a recently characterised GABAergic interneuronal type that has recently been described (Ibanez-Sandoval et al., 2011). These interneurons express NPY, have distinct electrophysiological properties and elicit large, long-lasting GABAergic IPSPs in MSNs (English et al., 2011). LTSIs also express NPY, therefore we have used NPY-EGFP positive BAC transgenic mice (see methods) to test the effect of firing in these neurons upon corticostriatal transmission. In order to discern LTSIs from NGFIs in striatal GFP expressing neurons, the electrophysiological properties of these neurons in these mice have been tested.

	LTSI	NGFI
Rebound spikes	19/19	0/17
Mean resting Vm (mV)	spont. active	-58.6±0.9
Mean Input resistance (MΩ)	967±106	320±34
Mean Ih ratio	1.00±0.02	0.93±0.07
Mean AHP (mV)	12.4±0.7	16.7±1.0
Mean mice age (days)	18.3	18.1

Table 4.1: Electrophysiological properties of tested NPY expressing neurons in NPY-EGFP BAC transgenic mice. We initially classified neurons based upon presence of rebound potentials and high input resistance (both signature properties of LTSIs), however we found that other properties were similarly divergent.

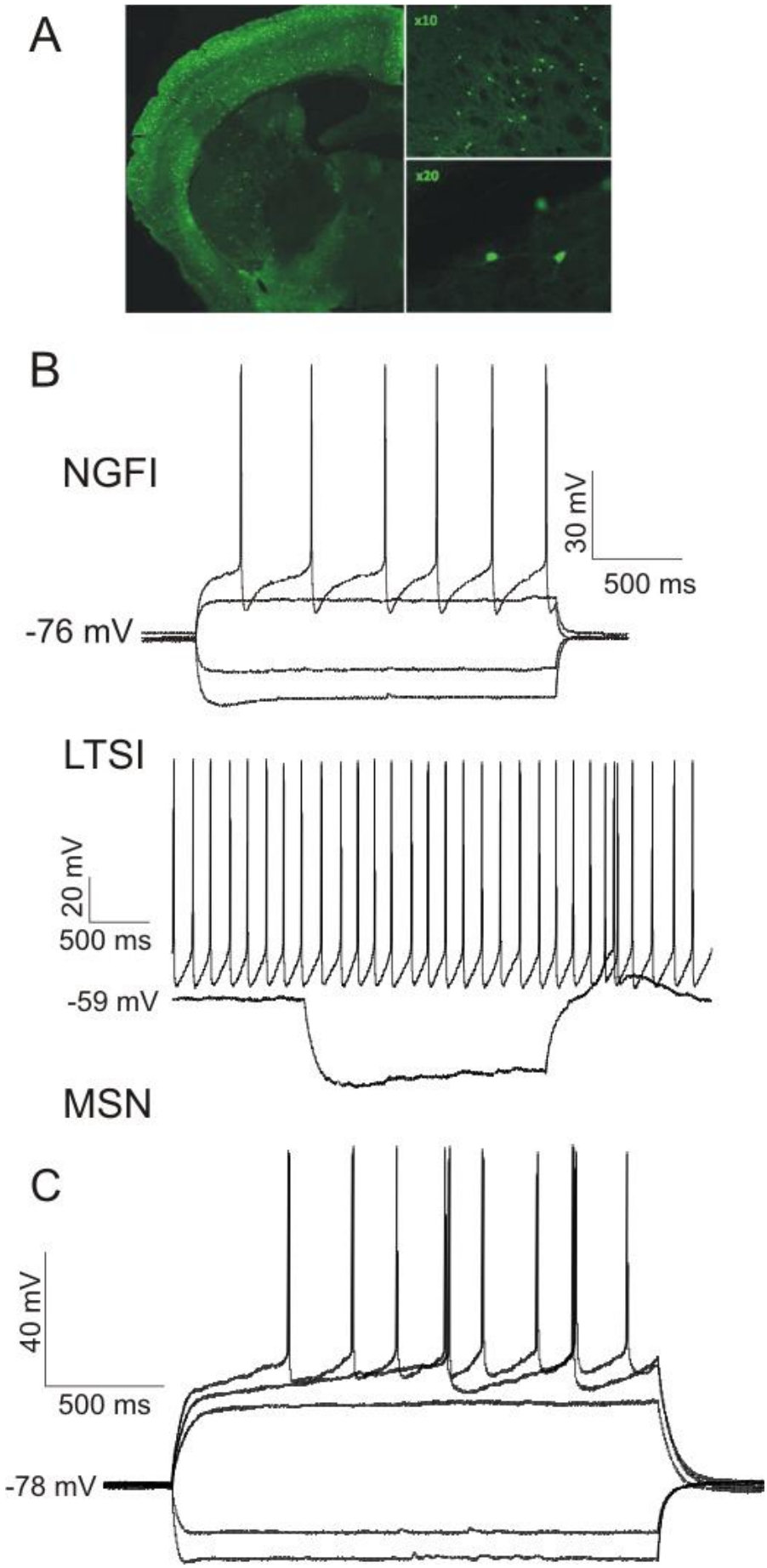
A
x10
x20
B
NGFI
30 mV
500 ms
-76 mV
LTSI
20 mV
500 ms
-59 mV
MSN
C
40 mV
500 ms
-78 mV

Figure 4.1: Electrophysiological properties of neurons in NPY-GFP expressing mice. (A) Coronal slice image of GFP fluorescence from NPY-GFP expressing BAC transgenic mice. In the striatum, GFP-expressing neurons are either LTSIs or NGFIs. **(B)** Responses of NGFI (top) and LTSI (bottom) in response to current steps. Note the larger AHPs in NGFIs and the pronounced Ih and rebound spikes in LTSIs. **(C)** Responses of MSN to current steps. MSN properties are similar to those reported in rat and wild-type CD57 mice.

The properties from neurons we have tested are listed in table 1. In agreement with results published by Ibanez-Sandanoval et al. (2011), we found 2 discrete subtypes in these neurons. One subtype was characterised by high input resistance, small AHPs, and rebound spikes following offset of hyperpolarising currents; properties characteristic of LTSIs. The other subtype was characterised by lower input resistance, larger AHPs and lack of rebound spikes; these correspond to properties of NGFIs described in Ibanez-Sandanoval et al. (2011). Our data do not correspond exactly with those in their publication. For example, they describe input resistances of LTSIs and NGFIs as 744±51 MΩ and 142±13 MΩ respectively, somewhat lower than our values. They also describe larger AHPs in both neuron types. Furthermore, all our LTSIs were spontaneously active, compared to only 75% in Ibanez-Sandanoval. However across all described properties, we observed similar subgroups of properties between putative LTSI and NGFI neurons which preserved the relative differences between these two neuron types. Responses to current steps are shown in Figure 4.1 B.

4.2.2 Striatal MSNs in NPY-positive mice express properties similar to wild type

As in rat, we recorded from striatal MSNs in these animals. These neurons expressed similar properties to rat MSNs. In all cases, we saw slow depolarisation to first spike and marked inward rectification at hyperpolarised potentials (Figure 4.1 B) In 13 neurons (from mice of average age 18.4 d), we recorded mean resting membrane potential of -77.8±1.2 mV, mean input resistance of 190±29 MΩ and AHPs of 11.4±0.8 mV. These figures are similar to our observations in rat (section 3.2.1). Other groups have reported similar resting membrane potential in mice but lower values for mean input resistance (Gertler *et al.*, 2008; Planert *et al.*, 2013). These studies report IRs of 53.1 MΩ for D1-expressing and 93.1 MΩ for D2-expressing MSNs and 90.6 MΩ for MSNs generally. However these studies use mice of older age than in our own experiments (P35-45 and P21-32 respectively) and neuronal IR is known to decrease with age which may account for the discrepancy (Oswald & Reyes, 2008).

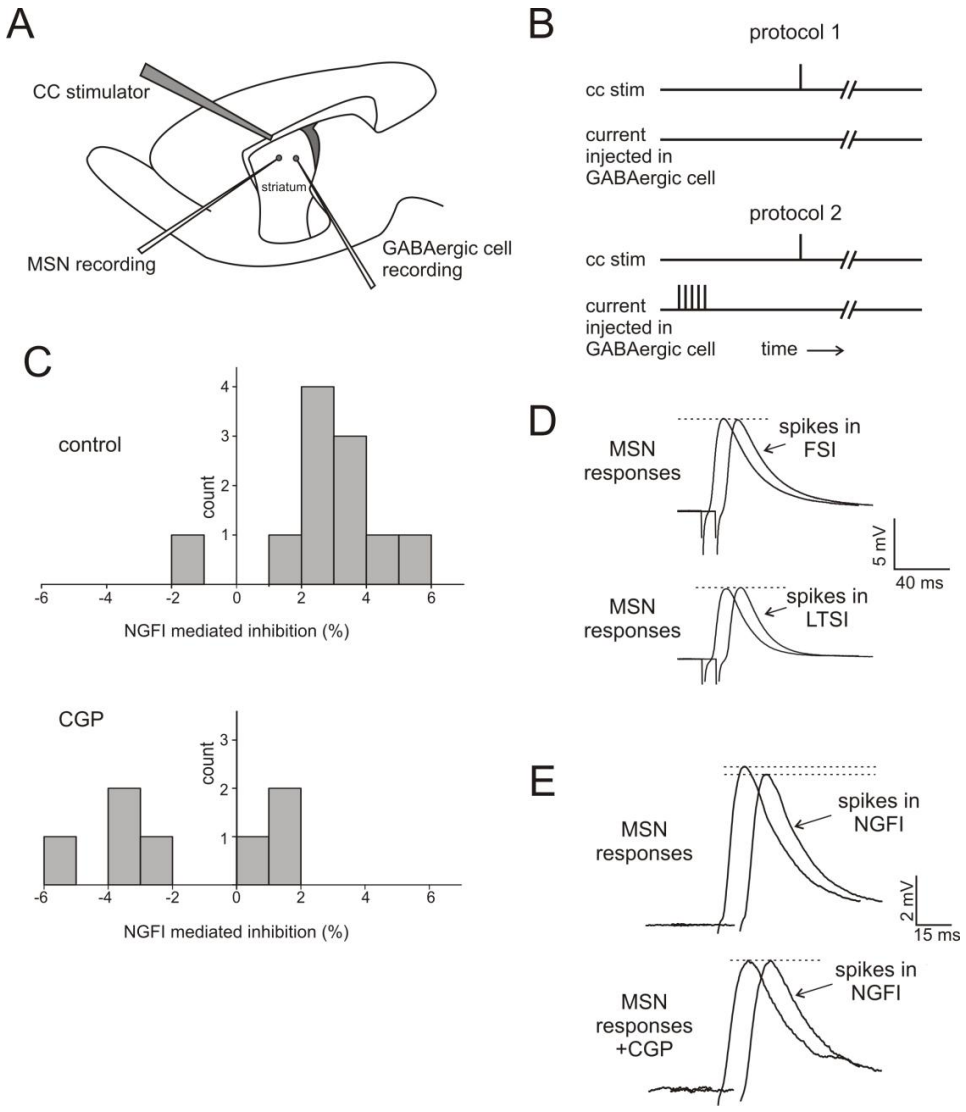

Figure 4.2: Individual NGFIs inhibit corticostriatal responses via $GABA_B$ receptors. (A) Positioning of recording and stimulating electrodes in dual recording experiments. An MSN and a second GABAergic neuron were recorded in the dorsolateral striatum. The distance between the two recorded cells was <100 µm. Corticostriatal fibres were activated by stimulation in the CC. **(B)** Stimulation sequence. Similar to Figure 1B, two stimulation protocols were applied consecutively. In the first protocol (10 s duration) a single CC stimulus was delivered. In the second protocol (bottom, 10 s duration), a single CC stimulation was preceded by five short (5 ms, 50 Hz) depolarising current pulses in the GABAergic cell, each of which elicited one action potential. This two-protocol cycle was applied without interruptions at least 75 times for each pharmacological condition. **(C)** Distribution of the inhibitory effect of NGFI action potentials on cortical responses of MSNs from 11 experiments in control solution (top), in 7 of which neuron longevity was sufficient for subsequent application of CGP 52432 (bottom). **(D)** Two representative examples of the lack of effects of spikes in FSIs and LTSIs on the CC-evoked responses of neighbouring MSNs. In the FSI-MSN experiment (top), each trace is the average of 121 consecutive MSN responses to a CC stimulus either preceded or not preceded by spikes in the FSI. In the LTSI-MSN experiment (bottom, different animal), each trace is the average of 57 consecutive presentations of one of the two protocols. **(E)** Average traces from a representative experiment. In control solution, MSN responses to CC stimulation were significantly ($P<0.05$) inhibited by preceding NGFI action potentials. In CGP 52432, this inhibition was abolished.

4.2.3 GABA released by single neurogliaform interneurons but not other GABAergic interneurons inhibits cortical glutamate release

In addition to MSNs, GABA is released by three well characterised types of interneurons in the striatum: LTSIs, FSIs and NGFIs. In these experiments, every other CC stimulation was preceded by five spikes evoked in the GABAergic interneuron (see Methods). The interval between the first spike and the CC stimulus was 500 ms for all the experiments. The two consecutive stimulation protocols used are illustrated in Figure 4.2 A, B. In each individual experiment out of a total of 7 FSI-MSN paired recordings (carried out in rats), spikes in a FSI failed to affect the CC-evoked responses of MSNs significantly (spikes preceded by an FSI were 99±2% of control). A paired t-test of average responses across all neurons also showed no significant inhibition. We further carried out 24 LTSI-MSN paired recordings using NPY-reporting BAC mice. In each individual experiment, spikes in LTSIs failed to significantly affect the CC-evoked responses of the simultaneously recorded MSNs (on average, responses preceded by spikes in an LTSI were 99.7±1.0% of control). Once more, a paired t-test of the average responses across all neurons failed to show significant differences between control responses and responses preceded by spikes in LTSIs. Examples of the absence of effects of spikes in a neighbouring LTSI or an FSI on MSN responses are shown in Figure 4.2 D. We concluded that a burst of spikes in an individual FSI or LTSI is not sufficient to elicit $GABA_B$ receptor-dependent inhibition of glutamatergic responses of a neighbouring MSN.

We also conducted paired recordings from NGFI-MSN pairs in BAC mice to test for their ability to cause $GABA_B$ receptor-mediated inhibition of glutamate responses. In contrast to the other GABAergic neurons tested, we found that in 4/11 NGFI-MSN paired recording experiments, spikes in the NGFI significantly inhibited subsequent CC-evoked responses in MSNs (ANOVA followed by post-hoc Bonferroni t-test, $p<0.05$; Figure 4.2 C, E). On average, in these 4 cases, EPSP inhibition induced by NGFI spikes was 4.2±0.4%. A paired t-test of the average responses across all 11 neurons showed significant ($p<0.05$) inhibition of responses.

In 7 of these experiments, including all cases in which significant effects were observed in control solution, CGP 52432 was subsequently applied, abolishing any

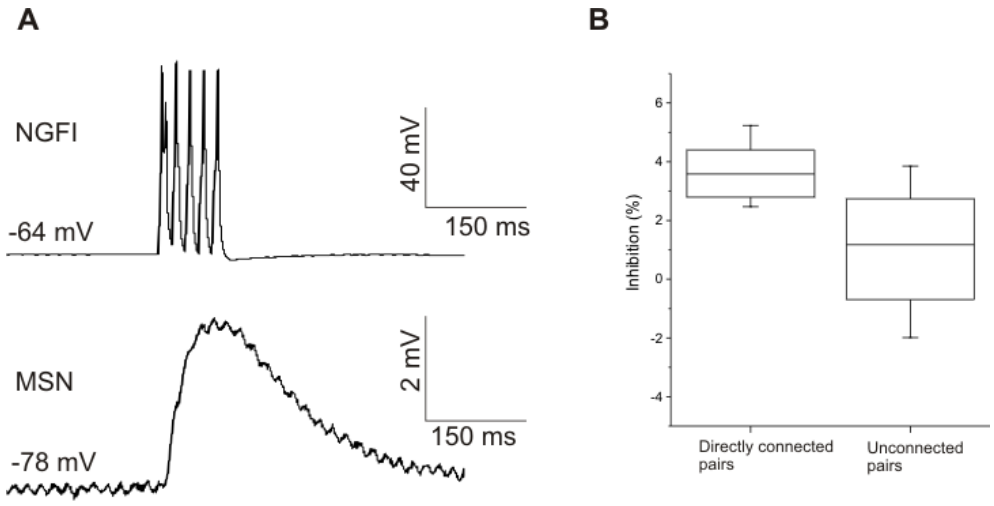

Figure 4.3: NGFI-MSN pairs with direct connections exhibit favourable corticostriatal inhibition. (A) Current clamp recordings of APs evoked in an NGFI by direct current injection (top) mediate $GABA_A$-dependent depolarisation in a nearby MSN (bottom). Depolarisation occurs as the resting Vm of the MSN is lower than ther reversal potential of $GABA_A$ receptors. Average of 11 traces. **(B)** Boxplot of corticostriatal $GABA_B$-dependent inhibition in pairs that exhibit or lack direct $GABA_A$-dependent connections. Directly connected pairs show marked more inhibition than unconnected pairs.

NGFI-mediated inhibition (Figure 4.2 C, E). We concluded that NGFIs are the only GABAergic neurons in the striatum capable of *individually* triggering $GABA_B$ receptor-mediated inhibition of glutamate responses through a burst of spikes.

We also tested connectedness between pairs NFI-MSN pairs. In the same 11 NGFI-MSN pairs, a train of 5 APs evoked in a patched NGFI showed direct connections in 6/11 pairs (Figure 4.3). In all 6 cases, IPSPs were inhibited by application of picrotoxin. From these two groups, we saw significant differences in NGFI-mediated $GABA_B$-dependant inhibition between connected and unconnected pairs. Of 6 directly connected pairs, 4 showed significant inhibition (average of 6 neurons 3.7±0.4%). In contrast, from 5 unconnected pairs, none exhibited significant inhibition (average of 5 neurons 1.0±1.1%). These findings suggest that direct connections between neurons are an important indicator of ability to inhibit presynaptically. Connected neuron pairs showed significantly (t-test, $p<0.01$) more inhibition than unconnected pairs.

4.2.4 NPY suppresses corticostriatal transmission

The role of NPY in striatal circuits has not been thoroughly investigated. Using bath application of NPY to superfused slices undergoing cortical stimulation, we investigated whether NPY could modulate corticostriatal transmission. Experiments similar to these have been conducted previously to investigate the role of other neurotransmitters at corticostriatal synapses (Nisenbaum *et al.*, 1992, 1993; Blomeley & Bracci, 2008). In our experiments, in the presence of $GABA_A$ and $GABA_B$ receptor antagonists, cortical fibres were stimulated every 10 s and responses recorded in MSNs via whole-cell patch-clamp (Figure 4.4 A,B). After at least 100 responses, NPY was bath applied. In a subset of experiments, NPY was washed.

In 3/3 experiments, MSN responses to CC stimulation were significantly (ANOVA followed by post-hoc t-tests with Bonferroni correction) reduced to 76.3±5.5% of control values in the presence of NPY (Figure 4.4 C, D, E). A paired t-test of response averages across the 3 experiments showed that NPY significantly ($p<0.05$) reduces responses to cortical stimulation. In 2 of these experiments an NPY wash was conducted, each neuron showing significant ($p<0.001$) recovery of responses in both neurons. After wash, responses were 90.0±1.3% of control responses (Figure 4.4 C, D, E). We also measured input resistance using small negative current steps. In the presence of NPY, input resistance increased to 107.5±3.2% of control values in 3 experiments. A paired t-test did not show significant change in IR ($p=0.071$). Upon wash of NPY, input resistance continued to increase to 113.3±4.1% of control responses. In one of these experiments, we used a paired pulse paradigm with a second pulse 10 ms after the first. Upon examining the paired pulse ratios (PPRs), we found that PPR increased from 0.84 to 0.88.

4.2.5 Sustained depolarisation of LTSIs suppresses corticostriatal transmission

LTSIs are the only striatal neurons that release NO (Vincent *et al.*, 1983; Kubota *et al.*, 1993). NO has been shown to be involved in LTD in corticostriatal synapses (Calabresi *et al.*, 1999c; Sergeeva *et al.*, 2007; Sammut *et al.*, 2010) and its function as a neurotransmitter generally is well known

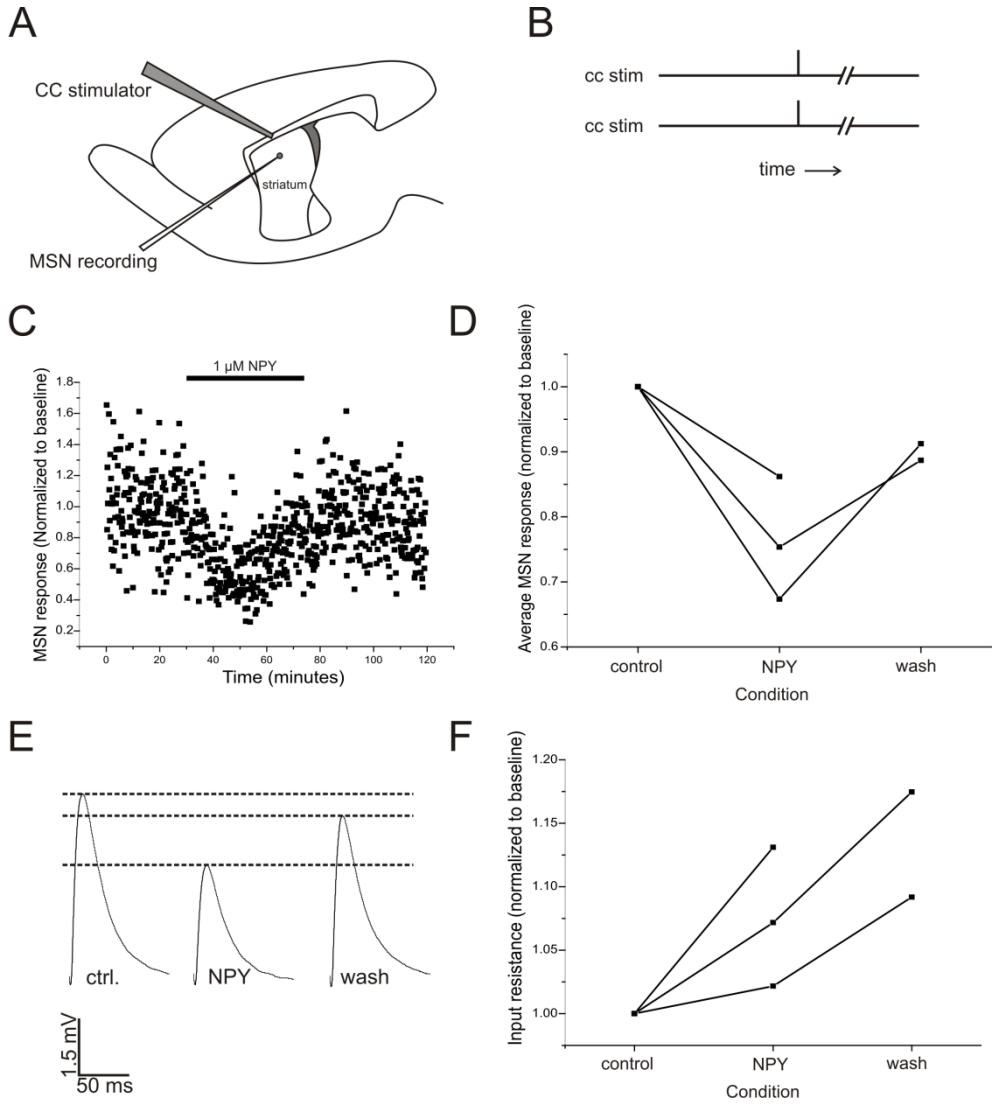

Figure 4.4: NPY suppresses corticostriatal transmission. (A) Positioning of recording and stimulating electrodes. An MSN was recorded in the dorsolateral striatum. Corticostriatal fibres were activated by stimulation in the CC. **(B)** Stimulation sequence. A simple protocol in which a single stimulation in the CC evoked responses in a patched MSN. This protocol was repeated every 10 s. After control responses were recorded, NPY was added for comparison. NPY was washed in a subset of experiments. **(C)** Scatterplot of an experiment showing the MSN response to NPY and recovery upon wash. **(D)** Normalized line plot from 3 experiments. Each line represents a single experiment. All experiments showed a significant ($p<0.01$) reduction in MSN response after bath application of NPY. In 2 experiments where NPY was washed, responses recovered significantly ($p<0.001$). **(E)** Averaged traces of MSN responses from same experiment shown in (D). Control, NPY and wash conditions are averages of 179, 171 and 218 traces respectively. **(F)** Normalized line plot of IR changes from 3 experiments. Each line represents a single experiment. Each experiment showed a gradual increase in IR.

(Boehning & Snyder, 2003; Garthwaite, 2008). We have tested the transient effects of NO upon corticostriatal transmission. NO release is highly dependent upon NMDA receptor activation (Garthwaite, 2008) which is strongly evoked at depolarised potentials (Cherubini *et al.*, 1988; Kita, 1996; Logan *et al.*, 2007). For example, NMDA-dependent EPSPs in striatal neurons have been shown to be significantly enhanced at membrane potentials above -50 mV (Cherubini *et al.*, 1988). Therefore, in the presence of $GABA_A$ and $GABA_B$ receptor antagonists, we elicited 500 ms depolarisations in a patched LTSI to facilitate endogenous NO synthesis and release.

We concurrently evoked glutamatergic EPSPs in patched MSNs by stimulation in the CC (Figure 4.5 A, B). LTSI depolarisation preceded CC stimulations by 0.75 and 10.75 s (Figure 4.5 A, B). In 4/5 experiments, LTSI depolarisations significantly inhibited CC-evoked EPSPs at either the 0.75 s or 10.75 s interval (ANOVA with post-hoc t-tests with Bonferroni correction). Across all 5 experiments, CC-evoked EPSPs were singnificantly (paired t-tests of average responses) reduced by 6.4±1.8% ($p<0.05$) at 0.75 s interval and 6.2±1.0% ($p<0.01$)) at 10.75 s interval (Figure 4.5 C, E). Whilst each time interval was significantly different from control, time intervals were not significantly different from each other. In 4 of these experiments, we tested input resistance in each condition (Figure 4.5 D) using small negative current steps. These experiments did not show a consistent change in input resistance, which wou. Across 4 experiments where significant changes in cortical responses were seen, input resistance was 0.99±0.01 of control at 0.75 s interval and 1.00±0.02 at 10.75 s interval (ranges: 0.98-1.02 and 0.96-1.07).

4.3 Discussion

The main finding of this chapter is that single NGFIs but not LTSIs or FSIs can reduce cortical input onto MSNs via $GABA_B$ receptors. We have also shown that LTSIs can reduce corticostriatal transmission through a non-GABA mechanism and that exogenous NPY suppresses corticostriatal transmission.

We first characterised the properties of NPY-EGFP expressing striatal neurons (table 4.1, Figure 4.1). Our results showed two distinct subgroups of neurons, consistent with that shown in the previous publication (Ibanez-Sandoval *et al.*,

2011). In particular, differences in input resistance and prevalence of rebound spikes were marked. Whilst our data do not correlate exactly with their data, the relative difference between the properties of putative LTSIs and NGFIs was preserved. We tested animals with average age of 18 days compared to 2-3 months by Ibanez-Sandanoval et al. The postnatal period up to 25 days has been shown to be critical for development of properties of striatal neurons (Tepper & Trent, 1993; Szele *et al.*, 1994; Butler *et al.*, 1999; Chesselet *et al.*, 2007) therefore the younger age of animals tested here may contribute to the discrepancy.

Our observed properties for LTSIs also differ from those recorded in rats. Input resistances were higher and AHPs lower in mouse LTSIs than in rat. This may reflect differing development of various properties between these two species.

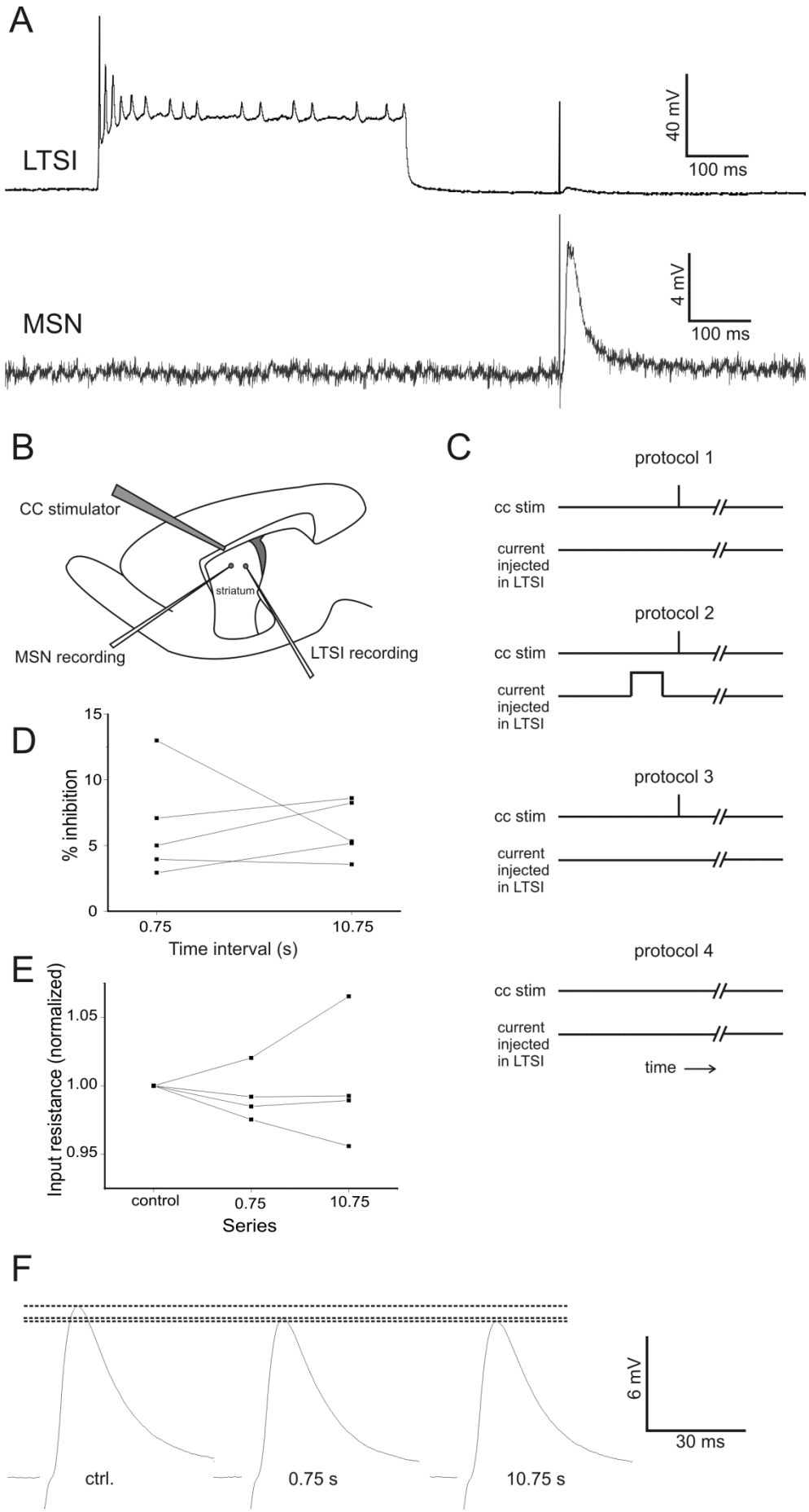
A
LTSI
40 mV
100 ms
MSN
4 mV
100 ms
B
CC stimulator
striatum
MSN recording
LTSI recording
C
protocol 1
cc stim
current injected in LTSI
protocol 2
cc stim
current injected in LTSI
protocol 3
cc stim
current injected in LTSI
protocol 4
cc stim
current injected in LTSI
time
D
% inhibition
15
10
5
0
0.75
10.75
Time interval (s)
E
Input resistance (normalized)
1.05
1.00
0.95
control
0.75
10.75
Series
F
6 mV
30 ms
ctrl.
0.75 s
10.75 s

Figure 4.5: Sustained depolarisation of LTSIs suppresses corticostriatal transmission. **(A)** Example trace showing long LTSI depolarisation beginning 0.75 s before CC stimulation. **(B)** Positioning of recording and stimulating electrodes. An MSN and LTSI were patched in the dorsolateral striatum. Corticostriatal fibres were activated by stimulation in the CC. LTSIs were depolarised by direct current injection **(C)** Stimulation sequence. Each protocol lasted 10 s. In protocol 1, CC stimulation provided a control response in an MSN. In protocol 2, CC stimulation was preceded by a 500 ms depolarisation of LTSI beginning 750 ms before CC stimulation. Protocol 3 was similar to protocol 1, however it provided information regarding effects 10.75 s after LTSI depolarisation. Protocol 4 was a 10 s rest period. These protocols cycled continuously for the duration of the experiment. **(D)** Line plot of experiments showing inhibition 0.75 s and 10.75 s after LTSI depolarisation. Each line is a single experiment. All experiments showed significant inhibition at one or both intervals. **(E)** Normalized line plot of input resistance from 4 experiments. Each line represents a single experiment. No consistent pattern was seen across the four experiments. **(F)** Averaged traces of MSN responses from a representative experiment. Control, 0.75 s and 10.75 s interval traces are an average of 40, 40 and 39 frames respectively.

The recently characterised NGFIs elicit large and long-lasting $GABA_A$ receptor-mediated IPSPs in MSNs (Ibanez-Sandoval et al., 2011; English et al., 2012), consistent with a strong release of GABA from their terminals. The present results suggest that they also cause an even slower presynaptic inhibition of the excitatory inputs to MSNs.

Several possibilities exist for the precise microcircuit responsible for these results. These are: 1) GABA spillover from NGFI-MSN synapses into cortical-MSN synapses; 2) Direct axo-axonal synapses from NGFIs onto the presynaptic bouton of cortical axon terminals; 3) NGFI axon terminals releasing GABA into the extracellular space by volume transmission. Our results show that NGFIs with direct GABAergic connections to MSNs are significantly more likely to inhibit corticostriatal transmission (Figure 4.3). This suggests that NGFI-mediated inhibition, like that of lateral MSN-MSN connections, occurs via GABA spillover from NGFI-MSN synapses to corticostriatal synapses. Furthermore, no axo-axonal synapses received by cortical axon terminals in the striatum have been reported.

While the effects caused by spikes in a single NGFI were relatively small, many such interneurons are in the position to affect the input to an MSN (Ibanez-Sandoval et al., 2011); furthermore, the all-or-nothing nature of spike generation means that the ability of a glutamatergic input to drive an MSN above threshold may be impaired even by a small reduction in glutamate receptor conductances. It is also possible that the 500 ms interval does not produce peak inhibition in this

manner. Whilst it is unlikely that longer intervals would show stronger inhibition (since MSN-MSN inhibition decayed after this interval) we cannot rule out the possibility that shorter intervals produce a stronger effect.

Thus, NGFIs are in a position to exert a strong influence on the local striatal circuits. It will be important from a functional point of view to determine the nature of the glutamatergic inputs that these interneurons receive from the cortex and the thalamus.

We have also shown that exogenous application of NPY can suppress corticostriatal transmission (Figure 4.4). Although only 3 experiments have been undertaken, our results strongly suggest that NPY can inhibit corticostriatal input. Similar to exogenous application of 1 µM baclofen, which suppresses cortical input by 65±22% (Nisenbaum *et al.*, 1992) and which we have demonstrated in Figure 3.3, exogenous application of 1µM NPY reduced cortical input onto MSNs by 24±5% (Figure 4.4 D, E). We further showed that wash of NPY ameliorates cortical responses to 90±1% of control values (Figure 4.4 D). No correlation was seen between changes in input resistance and presence or absence of NPY, suggesting that NPY-dependant inhibition was not mediated postsynaptically.

The simplest explanation for these results is that NPY is acting directly at corticostriatal synapse. The question of the endogenous source of this effect remains unanswered. Our previous experiments with NGFIs showed that, in the presence of $GABA_A$ and $GABA_B$ receptor antagonists, NGFIs do not modulate corticostriatal transmission (Figure 4.2) suggesting that if the source is the striatal NGFI population, single NGFIs are incapable of activating these receptors. Therefore, synchronous release of NPY by several NGFIs may be necessary. LTSIs are a second possibility however little is known about the release of NPY from these neurons.

Whilst a direct effect of NPY on these synapses is the most straightforward explanation, we cannot rule out NPY acting upon other striatal neurons to mediate this effect in our experiments. For example, Y2 receptor agonists have been shown to facilitate dopamine release in the striatum (Adewale *et al.*, 2007) and dopamine can suppress glutamate release at corticostriatal synapses via presynaptic D2

receptors (Bamford *et al.*, 2004a; Bamford *et al.*, 2004b). It will be necessary to conduct further experiments in order to explore these possibilities.

Finally, we have tested the effect of long LTSI depolarisations upon corticostriatal glutamate release (Figure 4.55). Whilst depolarisations of this kind have not been shown to be physiological, this protocol allows preferential evocation of endogenous NO release. LTSIs form synapses with the distal dendrites of MSNs, however the GABAergic IPSPs produced are weak and/or sparse, especially in comparison to the robust feedforward inhibition mediated by FSIs and NGFIs (Partridge *et al.*, 2009; Gittis *et al.*, 2010). Therefore it has been speculated that the primary role of LTSIs in striatal function is modulatory and mediated by non-GABAergic transmitters. Our long depolarisation protocol inhibited glutamate release over long periods of up to 10.75 s after LTSI depolarisation (Figure 4.5 C, E). Our experiments also showed no clear effect upon input resistance of the patched MSN (Figure 4.5 D), suggesting this activity is presynaptic. While sustained depolarisation in this manner is unlikely to occur naturally, this protocol produces endogenous release of NO which is difficult to evoke through other methods

Although this protocol preferentially releases NO, and while the long lasting effects would suggest nitrergic activity, we cannot rule out the possibility that these effects were mediated by NPY or SSN. SSN has been shown to presynaptically inhibit GABA release at MSN-MSN synapses (Lopez-Huerta *et al.*, 2008) and modulate K_{Ca} currents in MSNs (Galarraga *et al.*, 2007). However the expression of SSN receptors at corticostriatal terminals is unknown. Furthermore, as we have shown in Figure 4.4, NPY can inhibit corticostriatal transmission. Further experiments will be essential to elucidate the mechanisms underlying these effects.

Chapter Five: General discussion

This thesis has focused upon the corticostriatal synapse and how the action of striatal neurons regulates this important connection. The presented results shall be summarised and discussed in the wider context of basal ganglia function. It shall also examine the scientific and technical limitations of the work undertaken and suggest avenues for future investigation.

5.1 Medium spiny neurons presynaptically inhibit corticostriatal transmission through $GABA_B$ receptors

Understanding the convergence of cortical projections onto MSNs is of critical importance to understanding BG function. Cortical pyramidal neurons, which convey information pertaining to movement, sensory and associative function, impart this information to MSNs through these projections (Wilson *et al.*, 1983; Ingham *et al.*, 1993). How do neurons of the striatum process this information? What are the functional interactions?

The lateral connections between MSNs have been postulated to be important in the "winner-take-all" model of basal ganglia function. In this model, competing cortical inputs "bid" for movement responses; when an MSN or a group of MSNs fire, their lateral connections suppress surrounding MSNs, reinforcing the positive striatopallidal signal (Jaeger *et al.*, 1994; Tunstall *et al.*, 2002; Plenz, 2003; Tepper *et al.*, 2008; Gittis *et al.*, 2010). However, these lateral interactions have been shown to be relatively weak, especially in comparison to the inhibitory action of FSIs and NGFIs (Jaeger *et al.*, 1994; Czubayko & Plenz, 2002; Tunstall *et al.*, 2002; Gittis *et al.*, 2010; Ibanez-Sandoval *et al.*, 2011). Our experiments presented here demonstrate that MSNs can suppress glutamate release onto nearby MSNs from cortical terminals. We have shown that this inhibition is mediated by presynaptic $GABA_B$ receptors and that a time interval 500 ms after GABA release inhibits more strongly than at longer intervals. However, even after 2 s, significant inhibition remains. Presynaptic $GABA_B$ receptor activation inhibits the activity of Q-type Ca^{2+} channels which in turn limits vesicular exocytosis of glutamate (Barral *et al.*, 2000).

Whilst a single MSN was incapable of inhibiting cortical input, firing by a group of MSNs stimulated antidromically in the globus pallidus inhibited cortical input.

These data reinforce previous research that has shown that MSNs can modulate glutamate release through the action of the co-transmitters substance P and enkephalin (Blomeley *et al.*, 2009; Blomeley & Bracci, 2011). Importantly, substance P and enkephalin-mediated modulation required the activity of only a single MSN, whereas we have shown that $GABA_B$-mediated effects require synchronous activity from a group of MSNs. Such synchronous activity has been shown to occur in response to bursts of cortical stimulation (Carrillo-Reid *et al.*, 2008). Furthermore, we have shown that $GABA_B$-mediated effects are maximal at 500 ms intervals and continue until 2 s. In comparison, enkephalin-mediated inhibition exhibited a similar timecourse, whereas substance P-mediated facilitation peaked after only 250 ms intervals and was shown to decay after only 1 s. $GABA_B$-mediated effects were also relatively weaker. At 500 ms intervals, GP stimulation reduced CC-evoked EPSPs by 10.0±0.8% in significant neurons. In comparison, μ-opioid receptor dependant inhibition reduced EPSPs by 29.6±11.4% (Blomeley & Bracci, 2011) and individual MSNs facilitated EPSPs by 12.5±3.7% through action of substance P (Blomeley *et al.*, 2009). In some experiments, it was clear that GP stimulation produced no inhibition. In these experiments, it may have been the case that the GP stimulator was simply not positioned in an optimum location to stimulate striatal MSNs.

Collectively, these data are significant in the context of the competitive dynamics of striatal action selection and recognition of patterns of cortical activity (Redgrave *et al.*, 1999; Tunstall *et al.*, 2002). Currently, opinions differ as to the relative importance of the weak direct GABAergic interactions between MSNs (Wickens *et al.*, 2007; Wilson, 2007). Whilst inhibition shown by our data is relatively small, the principles of AP generation show that only a small reduction in EPSP may be sufficient to prevent a postsynaptic neuron from depolarising the the AP threshold potential. Therefore, in light of the $GABA_B$-meditated effects revealed here, it will be important for existing models of striatal function to be updated to reflect the increased feedback inhibition mediated by MSNs upon other cortical inputs.

Many unanswered questions remain. These include the relative interactions between striatonigral and striatopallidal MSNs. Stimulation in the GP evokes antidromic APs in similar numbers of MSNs from the two pathways (Lopez-Huerta *et al.*, 2013). Therefore, from our data, we cannot draw conclusions regarding the relative inhibitory characterisitics of D1-expressing or D2-expressing MSNs in this microcircuit. Substance P is released by D1-expressing MSNs only and enkephalin by D2-expressing MSNs only, however both their effects are mediated equally

upon MSNs of both subtypes (Blomeley *et al.*, 2009; Blomeley & Bracci, 2011). In contrast, GABA is released by neurons of both pathways .Given that each pathway synapses with both direct and indirect pathway MSNs at similar levels (Yung *et al.*, 1996) and the non-specificity of substance P and enkephalin-mediated effects, it is not unreasonable to speculate that GABA effects described here are also non-specific in their target pathways. However, further experiments will be required to confirm this.

In these experiments, whilst it was likely that our stimulation protocols predominantly evoked APs in corticostriatal axon projections, the possibility that some thalamostriatal fibres were activated cannot be entirely ruled out. Thalamic parafascicular nuclei neurons emit projections that terminate in the striatum but project via the motor cortex or GP (Deschenes *et al.*, 1996a), while individual central lateral thalamic nuclei neurons project to both cortex and thalamus (Deschenes *et al.*, 1996b). However, we are confident that our sitmulation protocols predominantly evoke glutamate realease from cortical axon terminals due to the high density of these fibres and due to our preparation selectively preserving these projections at the expense of thalamic projections.

Whilst the expression of various receptors at thalamostriatal synapses is known (Bradley *et al.*, 1999; Kosinski *et al.*, 1999; Charara *et al.*, 2000; Kieval *et al.*, 2001; Paquet & Smith, 2003), little work has been undertaken on the modulation of these terminals by exogenous or endogenous ligands. MSNs receive similar numbers of thalamic and cortical inputs, with thalamostriatal synapses important in excitation of some interneurons (Smith & Bolam, 1990a; Lapper & Bolam, 1992; Sadikot *et al.*, 1992; Doig *et al.*, 2010). Therefore investigation of the modulation of these inputs by striatal neurons in experiments similar to those described here would be desirable.

5.2 NPY and NPY-expressing neurons modulate corticostriatal transmission

In our experiments, we have investigated the contribution of NPY-expressing neurons to modulation of corticostriatal inputs onto MSNs. Importantly, we have found that single NGFIs are capable of suppressing glutamate release at these synapses.

We first characterised the properties of NPY-expressing neurons of the striatum. We found two discrete subtypes: the well known LTSIs and the recently discovered NGFIs. Only three previous publications from two laboratory groups have reported upon the role of NGFIs in striatum (English *et al.*, 2011; Ibanez-Sandoval *et al.*, 2011; Luo *et al.*, 2013). These have shown that NGFIs mediate large, long lasting, $GABA_A$ receptor-meditaed inhibition of MSNs (English *et al.*, 2011; Ibanez-Sandoval *et al.*, 2011) and are under the control of nicotinic activation by cholinergic interneurons (English *et al.*, 2011; Luo *et al.*, 2013). Therefore, our investigation of this neuron's basic properties and function is further confirmation of its burgeoning importance.

Our experiments have shown that NGFIs, but not FSIs or LTSIs, can inhibit glutamate release at corticostriatal synapses and that this inhibition is mediated by $GABA_B$ receptors. We have further shown that the probability that an NGFI can inhibit in this fashion is dependent upon the presence of direct GABAergic interactions in NGFI-MSN pairs. These data suggest that NGFI-mediated inhibition occurs due to GABA spillover from NGF-MSN synapses to corticostriatal synapses, similar to inhibition described in section 5.1. Although the inhibition is relatively small (4.2±0.4%) the nature of AP generation is such that even a small reduction in an EPSP can be sufficient to prevent MSN firing. Furthermore, this phenomenon is in addition to the powerful direct inhibition produced by these neurons (Ibanez-Sandoval *et al.*, 2011).

NGFIs have been implicated both in feedforward inhibition and as a critical part of cholinergic control of MSN responses (English *et al.*, 2011; Ibanez-Sandoval *et al.*, 2011; Luo *et al.*, 2013). Previously, FSIs were thought to be the only striatal neuron that could mediate powerful inhibition of MSNs (Mallet *et al.*, 2005; Wilson, 2007;

Tepper *et al.*, 2008). In this way, they have been perceived to play a unique role in feedforward inhibition and models of information processing in the striatum (Bar-Gad & Bergman, 2001; Wilson, 2007; Tepper *et al.*, 2008). The recent publications implicating NGFIs in direct inhibition of MSNs and the data presented here describing indirect inhibition will necessitate a re-evaluation of feedforward inhibition and its role in the striatum. NGFIs have been shown to be excited by ACh acting upon nicotinic receptors, which allow LAIs undergoing burst-pause behaviour to control MSNs via these neurons (English *et al.*, 2011; Luo *et al.*, 2013). Our data have thus shown another avenue through which NGFIs mediate this microcircuit.

We have also conducted experiments using NPY. Whilst NPY has been important as a marker of LTSIs and NGFIs, there has been little investigation of the role of the neurotransmitter itself. We tested the effect of NPY upon corticostriatal transmission. Whilst only 3 experiments have been conducted, our results strongly suggest that NPY can suppress corticostriatal transmission. A 1 µM bath application of NPY reduced cortically evoked EPSPs in patched MSNs to 76.3±5.5% of control values, which recovered to 90.0±1.3% of control responses upon wash. Our data also suggest that this action is presynaptic. Whilst this is an interesting finding, it raises more questions than it answers. What is the endogenous source, if any, of this activity? What receptors are being acted upon? The most parsimonious explanation is that NPY is released by LTSIs or NGFIs and acts directly upon cortical axon terminals. Our experiments with NGFIs have shown that in the presence of $GABA_A$ and $GABA_B$ antagonists, single NGFIs do not modulate this activity. However, this does not preclude the possibility that a group of NGFIs acting in concert may be sufficient to exert peptidergic control. LTSIs are the other NPY-releasing neuron but little is known of their activity in this respect.

It is also possible that NPY released by these neurons may act upon other areas of the striatum in order to mediate a disynaptic function. NPY has been shown to facilitate dopamine release in the striatum (Adewale *et al.*, 2007) and dopamine can suppress glutamate release at corticostriatal synapses via presynaptic D2 receptors (Bamford *et al.*, 2004a; Bamford *et al.*, 2004b). Therefore it will be important to clarify the role of NPY with further experiments.

We have also conducted experiments using long depolarisation of LTSIs, a protocol which preferentially releases NO (Garthwaite, 2008). LTSIs form synapses

upon MSNs, however these synapses are distal and sparse, resulting in weak GABAergic IPSPs compared to the stronger inhibition mediated by FSIs and NGFIs (Partridge *et al.*, 2009; Gittis *et al.*, 2010; Ibanez-Sandoval *et al.*, 2011). Therefore it has been speculated that the primary role of LTSIs in striatal function is modulatory and mediated by its non-GABAergic transmitters.

Our experiments have shown that 500 ms depolarisations of LTSIs can inhibit corticostriatal transmission up to 10.75 s after start of depolarisation. Our data also failed to show any consistent effect upon input resistance. These experiments are the first evidence of LTSI phasic modulation of cortical input onto MSNs. While this protocol preferentially releases NO, and while the long lasting depolarisations would suggest NO activity, we cannot rule out the possibility that other co-transmitters mediated this effect. As we have shown, NPY is capable of inhibiting corticostriatal transmission. SSN presynaptically inhibits lateral MSN-MSN synapses and can modulate K_{Ca} currents in these cells (Galarraga *et al.*, 2007; Lopez-Huerta *et al.*, 2008). MSNs also express mRNAs for all 5 SSN-Rs (Galarraga *et al.*, 2007).

Collectively, our data have shown that the synapse between cortical axons and MSN dendrites is subject to modulation from several actors in the striatum. In this way, a miscellany of endogenous neurotransmitters from multiple sources contributes to the fine control of glutamate release. Modulation of input to MSNs in this manner does not produce the same functional effect as direct inhibition. Compared to direct inhibition, presynaptic modulation presents differing arithmetic functions upon neuronal input-output paradigms. Direct inhibition produces additive or multiplicative operations upon a neuron that modulates its normal output from excitatory inputs. However, presynaptic modulation changes the probability of neurotransmitter release, which can alter the filtering characteristics of the synapse, ie by shifiting the synapse from acting as a low-pass filter to band-pass or vice versa (Abbott & Regehr, 2004; Silver, 2010). Therefore, further experiments and computational modelling will be necessary to learn more on striatal function (Gurney, 2007; Wickens *et al.*, 2007).

5.3 Future experimental work

Whilst the results presented here shed light upon striatal function, important questions are raised. Below, the most salient experiments that should be undertaken and the rationale behind them is summarised.

The optogenetic revolution of the last several years has opened new possibilities in the field of electrophysiology. In the striatum, it has been used to control populations of D1-expressing and D2-expressing MSNs, LAIs as well as cortical, thalamic and dopaminergic afferents (Kravitz *et al.*, 2010; Chuhma *et al.*, 2011; English *et al.*, 2011; Bepari *et al.*, 2012; Kravitz *et al.*, 2012; Bass *et al.*, 2013; Kravitz *et al.*, 2013; Kress *et al.*, 2013; Lenz & Lobo, 2013).

In Chapter 3, we investigated lateral inhibition of corticostriatal synapses by MSNs. Continuing these experiments, but replacing pallidal stimulations with optogenetic excitation of D1- or D2-expressing MSNs, would provide information on the relative contribution of these two subgroups. Patched MSNs could also be identified by their response to light. Furthermore, optogenetic activation would allow testing of intervals shorter than 500 ms. Compared to GP stimulation, optogenetic stimulation also allows greater numbers of MSNs to be stimulated simultaneously and of course, allows greater specificity (Chuhma *et al.*, 2011). Potentially, this may reveal stronger inhibition of cortical inputs than reported here. Similarly, the inhibitory action of NGFIs upon both direct- and indirect-pathway MSNs should be investigated.

Further to this, we have only tested NGFI-MSN pairs at a single stimulation interval. Whilst it is unlikely that inhibition peaks at intervals longer than 500 ms (since MSN-MSN inhibition decayed after this time period) shorter intervals may produce stronger effects. Furthermore, NGFI-mediated IPSPs are much longer than MSN-MSN IPSPs, therefore it would be interesting to explore the possibility that these effects extend beyond the 2 s interval we have shown here. Thus, experiments with multiple time intervals, featuring both shorter and longer intervals than 500 ms, should be undertaken to confirm the time course of NGFI-mediated inhibition.

Thalamic inputs also project to the striatum, in particular synapsing with cholinergic interneurons and MSNs (Smith *et al.*, 2004). Whilst receptor expression at these synapses has been described, little is known about the characteristics of activating these receptors by endogenous sources. Thalamostriatal synapses, unlike corticostriatal synapses, mainly form on the dendritic shaft of MSNs (Smith & Bolam, 1990a; Sadikot *et al.*, 1992; Jakab & Goldman-Rakic, 1996) therefore testing inhibition of glutamate release at these synapses would be interesting. Experiments similar to those we have conducted would investigate this important afferent tract. Similar to previous research (Ding *et al.*, 2010; Blomeley *et al.*, 2011), these experiments would stimulate in the reticular nucleus and would require horizontal slices, as these preserve thalamostriatal connections. An off-horizontal preparation has been shown to preserve both thalamostriatal and corticostriatal projections (Smeal *et al.*, 2007). Testing thalamic afferents using these kinds of experiments would also be possible with striatal interneurons.

The role of NPY in corticostriatal suppression has been shown here, however the identity of the endogenous factor(s) has not been revealed. Single NGFIs and LTSIs undergoing a burst of 5 APs were incapable of inhibition in the presence of $GABA_A$ and $GABA_B$ antagonists. However, several neurons working in synchrony may be sufficient to mediate this effect. At this time, optogenetic stimulation of NGFIs or LTSIs is not possible as varieties of transgenic mice that make this possible do not yet exist. However, experiments may be conducted that rule out disynaptic effects. Our experiments were only undertaken in the presence of picrotoxin, therefore it is possibly that NPY was acting upon other areas such as facilitating dopamine release. Therefore, these experiments should be repeated in the presence of additional antagonists including for dopamine, opioid and cholinergic antagonists. These experiments should also feature a paired pulse ratio protocol to determine if the action is presynaptic or postsynaptic.

We have shown that long depolarisations in single LTSIs can suppress cortical input. While these experiments preferentially release NO, this should be confirmed pharmacologically. NO acts upon sGC, therefore inhibitors of sGC such as H-[1,2,4]oxadiazolo[4, 3-a]quinoxalin-1-one (ODQ) could confirm NO-dependent effects. These experiments should be conducted in the presence antagonists of GABA-Rs and SSN-Rs. NO levels can also be measured through amperometric techniques (Berkels *et al.*, 2001; Boo *et al.*, 2011).

Further experiments may be conducted using multi-electrode arrays (MEAs). MEAs can record extracellularly from many neurons simultaneously (Spira & Hai, 2013). In conjunction with CC and GP stimulation, these may provide information on the spatial and temporal distribution of inhibitory effects by recording synptic signals at multiple electrodes as these signals propagate through the striatum.

5.4 Technical considerations

In this thesis, the main technique utilised has been single and paired patch recordings of neurons. For several decades this technique has produced invaluable information on the properties of cells and neuronal networks. Whole-cell recordings provide electrical access to the cell cytosome, allowing direct recording of the internal electrophysiological state (Sigworth & Neher, 1980; Hamill *et al.*, 1981; Sakmann & Neher, 1984). Compared to other methods, such as cell-attached or extracellular recordings, whole-cell provides the advantage of very accurate recordings. However this comes at the expense of potentially altering the natural state of the neuron through disruption of the cell membrane and dialysis of the internal pipette solution into the neuron (Sakmann & Neher, 1984). Furthermore, the pipette-membrane interface is by its nature fragile. In the case of our experiments, some of which lasted for several hours, it was essential that patched MSNs did not become damaged due to either of these aspects and we therefore closely monitored neuron state throughout our experiments, discarding those where neurons became damaged. In our case, the use of this technique was justified as it was essential not only to record inhibition of EPSPs that, in control, display wide variation but also as it permitted delicate control of stimulation of individual neurons.

Combining this technique with extracellular stimulation allows flexible control of stimulation protocols, allowing us to probe aspects such as the time course of neurotransmitter effects. Antidromic stimulation in particular allowed us to selectively excite MSNs in the striatum, as these are the only striatal neurons that project to this area. The disadvantage in our experiments was GP stimulation exerting small antidromic-orthodromic depolarisations in patched MSNs which must be allowed to subside before CC stimulation. This phenomenon prevented us from testing intervals shorter than 500 ms.

Combining these techniques resulted in other technical challenges. A major issue was patching MSNs that responded to both cortical input and GP stimulation/2nd patched neuron. For this reason, in FSI-MSN experiments and LTSI-MSN experiments where we have seen no GABAegic inhibition, we cannot be certain that this is due to a lack of the phenomenon existing at all or that simply that in our particular experiments, patched MSNs did not receive convergent afferents from both sources. Similarly, in experiments involving antidromic stimulation of multiple MSNs, it may be that experiments where inhibition was not seen was simply due to a lack of connectivity between GP stimulated MSNs and patched MSN. In paired patch experiments, concurrently patching two neurons that necessarily must be in close proximity also compounds the maintenance of whole cell patch integrity, as inserting a second pipette tip into a brain slice within 100 µm of an initial patch necessarily moves and distorts the surrounding tissue. Therefore great care was required when conducting these experiments.

We also saw issues surrounding slice visualisation. We necessarily had to maintain a balance between thinner slices that would show improved visualisation and thicker slices that would better preserve corticostriatal fibres. We also found that as animals aged, connectivity improved but visualisation deteriorated due to denser neuropil. For this reason, at younger aged animals (P14-19) we tended to use 250 µm slices whereas older animals (P20 and above) we used 200 µm slices.

5.5 Concluding remarks

The study of basal ganglia circuits has made great strides in the past few decades. Twenty years ago, the basic properties of some interneurons were only just being revealed (Kawaguchi, 1993). In recent decades the basic architecture of the basal ganglia has been unveiled (Wilson, 2004; Tepper *et al.*, 2007) and theories concerning action selection and striatal function have emerged (Alexander *et al.*, 1986; Redgrave *et al.*, 1999).

The most important issue concerning the BG is arguably the flow of information through this network and how information is processed. This thesis has provided its own contribution by unveiling novel interactions affecting striatal processing that will require incorporation into BG models. In future, the continued practical

research into and modelling of striatal and BG networks will provide new insights into function and new opportunities for the study of disease states.

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Appendix: Presynaptic control of corticostriatal synapses by endogenous GABA

Brief Communications

Presynaptic Control of Corticostriatal Synapses by Endogenous GABA

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Corticostriatal terminals have presynaptic $GABA_B$ receptors that limit glutamate release, but how these receptors are activated by endogenous GABA released by different types of striatal neurons is still unknown. To address this issue, we used single and paired whole-cell recordings combined with stimulation of corticostriatal fibers in rats and mice. In the presence of opioid, $GABA_A$, and NK1 receptor antagonists, antidromic stimulation of a population of striatal projection neurons caused suppression of subsequently evoked EPSPs in projection neurons. These effects were larger at intervals of 500 ms than 1 or 2 s, and were fully blocked by the selective $GABA_B$ receptor antagonist CGP 52432. Bursts of spikes in individual projection neurons were not able to inhibit evoked EPSPs. Similarly, spikes in fast spiking interneurons and low-threshold spike interneurons failed to elicit detectable effects mediated by $GABA_B$ receptors. Conversely, spikes in individual neurogliaform interneurons suppressed evoked EPSPs, and these effects were blocked by CGP 52432. These results provide the first demonstration of how $GABA_B$ receptors are activated by endogenous GABA released by striatal neuronal types.

Introduction

The striatum is the largest nucleus of the basal ganglia and is critically involved in motor control, action selection, and reinforcement learning (Alexander and Crutcher, 1990; Graybiel et al., 1994). Massive projections target the striatum from the cortex and the thalamus, forming glutamatergic synapses mainly on the dendrites of the medium spiny neurons (MSNs), the striatal projection cells (Bolam et al., 2000). As far as striatal neurons are concerned, GABA is by far the major neurotransmitter, being expressed by all MSNs and at least three well characterized classes of interneurons (Tepper and Bolam, 2004; Ibáñez-Sandoval et al., 2011). The axon collaterals of MSNs form symmetric GABAergic synapses with other MSNs. Paired recording experiments have shown that these synapses elicit $GABA_A$ receptor-mediated IPSPs in the postsynaptic neuron (Tunstall et al., 2002; Tepper et al., 2008). Fast spiking interneurons (FSIs), low-threshold spike interneurons (LTSIs), and neurogliaform interneurons (NGFIs), also form functional synapses with MSNs (Tepper et al., 2008; Ibáñez-Sandoval et al., 2011; Szydlowski et al., 2013). $GABA_B$ receptors are ubiquitous metabotropic receptors that mediate presynaptic and postsynaptic inhibition throughout the brain (Chalifoux and Carter, 2011). However, their role in mediating communication among striatal neurons is still poorly understood, as their activation by synaptically released GABA has not been demonstrated. In the striatum, $GABA_B$ receptors are found on GABA terminals, on glutamate terminals of cortical and thalamic origin and on the dendrites of MSNs (Lacey et al., 2005). Despite the presence of postsynaptic receptors, application of exogenous $GABA_B$ agonists does not produce measurable effects on MSN membrane properties. On the other hand, exogenous activation of $GABA_B$ receptors strongly suppresses glutamatergic inputs onto MSNs acting through a presynaptic mechanism (Calabresi et al., 1991; Nisenbaum et al., 1993). Whether and how these presynaptic $GABA_B$ receptors can be activated by endogenous GABA released by different striatal neurons remains to be established. Using protocols combining paired recording with stimulation of corticostriatal fibers, we have recently shown that an important modality of communication for the striatal neurons consists in the activation of presynaptic receptors located on glutamatergic terminals impinging on MSNs (Pakhotin and Bracci, 2007; Blomeley et al., 2009; Blomeley and Bracci, 2011). Here, we used similar procedures to unravel how presynaptic $GABA_B$ receptors are activated by different GABAergic striatal neurons.

Materials and Methods

Experiments were conducted on male and female P14–P21 Sprague Dawley rats and P14–P21 BAC transgenic mice heterozygous for the attachment of the NPY promoter to humanized renilla GFP (BAC-npy; stock no. 006417; The Jackson Laboratory). In these mice, NPY-GFP is expressed in the striatum by LTSIs and NGFIs (Partridge et al., 2009; Ibáñez-Sandoval et al., 2011). Rats and mice underwent cervical dislocation in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986. Following rapid brain removal, parasagittal slices (200–250-μm-thick) were cut using a vibroslicer (Campden Instruments) in a cutting solution of artificial CSF (ACSF; concentrations of the following in mM: 124 NaCl, 2 KCl, 1 $MgSO_4$, 1.25 KH_2PO_4, 2 $CaCl_2$,

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26 $NaHCO_3$, and 10 glucose) containing 1 mM kynurenic acid, maintained at 25°C and oxygenated with 95% O_2, 5% CO_2 gas. After cutting, slices were maintained for at least 30 min in ACSF without kynurenic acid. During recording, slices were superfused with ACSF (1.5–2 ml/min) and visualized using 10× and 40× water-immersion objectives using infrared and differential interference contrast microscopy.

NPY-expressing neurons were identified through epifluorescent excitation with a mercury lamp (Olympus U-RFL-T) in conjunction with standard GFP filters. Fluorescent neurons were identified as LTSIs or NGFIs based on their distinctive electrophysiological properties (Ibáñez-Sandoval et al., 2011). MSNs and FSIs were also identified based on their electrophysiological features (Bracci et al., 2003; Blomeley and Bracci, 2011).

Whole-cell recordings of striatal neurons were conducted using glass pipettes filled with intracellular solution (in mM: 120 K-gluconate, 20 KCl, 0.04 EGTA, 12 HEPES, 2 $MgCl_2$, 4 Na_2ATP, and 0.4 NaGTP, adjusted to pH 7.3 with KOH). Pipette resistance was 3–7 MΩ. Recordings were performed in current-clamp configuration using bridge amplifiers (BA-1S, BA-01×; NPI connected to a micro1401 analog–digital converted (5 kHz sampling) driven by Signal software. Input resistance was monitored with small negative current injections.

Single-recording experiments were conducted in the continuous presence of antagonists of the following receptors: $GABA_A$ (100 μM picrotoxin), D1 (10 μM SCH 23390 hydrochloride), D2 (3 μM S-(-)-Sulpiride), NK1 (10 μM L-732,138), opioid (10 μM naloxone hydrochloride), nicotinic (100 nM nicotine or 10 μM tubocurarine chloride), and muscarinic (25 μM atropine sulfate). Paired recording experiments involving two MSNs were conducted in the presence of $GABA_A$, opioid and NK1 receptor antagonists. Paired recording experiments from interneuron-MSN pairs were conducted in the presence of $GABA_A$ receptor antagonists only.

Glutamatergic EPSPs were evoked by electrical stimulation of the corpus callosum (CC), as in previous studies (Blomeley and Bracci, 2011). EPSPs were recorded from MSNs located in the dorsolateral striatum. In all experiments, a single CC stimulus was delivered continuously every 10 s. CC stimulation intensity was adjusted to produce EPSPs of 5–15 mV amplitude. After application of the $GABA_B$ receptor antagonist CGP 52432, the stimulation protocol continued, but the data acquired in the first 5 min following the start of the application were excluded from the analysis (to include only data acquired when the antagonist concentration had reached a steady state).

In single-recording experiments, every other CC stimulus was preceded by a train of stimuli (5 stimuli, 50 Hz) delivered by a second stimulator placed in the globus pallidus (GP) to activate antidromically MSN axons (Fig. 1*A*,*B*), as previously described (Blomeley and Bracci, 2009, 2011). This two-protocol cycle was applied without interruption at least 75 times for each pharmacological condition. The CC-evoked responses preceded by GP stimuli were then averaged and compared statistically with those not preceded by GP stimuli. The temporal interval between GP and CC stimulation was calculated from the first GP stimulus of the train to the CC stimulation. In some experiments, two intervals were tested; in this case a three-protocol cycle, comprising (2) no GP stimuli, (2) GP stimuli preceding CC stimulus by interval 1, and (3) GP stimuli preceding CC stimulus by interval 2, was continuously applied (at least 75 times per pharmacological condition). GP stimulation intensity was adjusted so that no antidromic spike was observed in the recorded MSN (Blomeley et al., 2009).

In paired recording experiments, a MSN and a second GABAergic neuron located within 100 μm were recorded simultaneously. Every other CC stimulus was preceded by a train of action potentials (5 spikes at 50 Hz) elicited by short (5 ms) current injections in the second neuron (see Fig. 3*A*), as in previous studies (Blomeley et al., 2009; Blomeley and Bracci, 2011). Again, this two-protocol cycle was applied without interruption at least 75 times for each pharmacological condition. The CC-evoked responses preceded by spikes in the other neuron were then averaged and compared statistically with those not preceded by spikes. Intervals were defined as time from the first spike of a burst to the subsequent CC stimulation.

Data were tested using Student's *t* test; average effects are expressed as mean ± SEM.

Results

Antidromic stimulation of MSNs inhibits cortical inputs onto MSNs

Electrical stimulation of the GP triggers antidromic spikes in both striatonigral and striatopallidal MSNs because the axons of these cells pass through, or terminate, in this region. These antidromic spikes then trigger orthodromic spikes in MSN axon collaterals, causing neurotransmitter release (Guzmán et al., 2003; Blomeley and Bracci, 2009, 2011; López-Huerta et al., 2013). We therefore stimulated the GP to evoke GABA release from MSNs and to test its effect on glutamate responses evoked by CC stimulation (Fig. 1*A*,*B*). These experiments were performed in the presence of antagonists for $GABA_A$, dopamine, opiate, NK1, and acetylcholine receptors (see Materials and Methods) to prevent unwanted activation of these receptors by GP stimulation and MSN firing. An interval (see Materials and Methods) of 500 ms was chosen, as it had previously been found to maximize opioid-mediated presynaptic inhibition (Blomeley and Bracci, 2011). In juvenile rats, GP stimulation significantly ($p < 0.05$) reduced the amplitude of responses to subsequent CC stimulation in 16/21 neurons (average inhibition 8.6 ± 0.8%; Fig. 1*C*,*D*). To test whether these effects depended on $GABA_B$ receptors, in 19 of these experiments (14 of which significant inhibitory effects were observed in control solution) we subsequently applied the specific antagonist CGP 52432 (1 μM), while continuing to apply the same stimulation protocol. In all cases, in the presence of CGP 52432, GP stimulation failed to cause significant inhibition of CC-evoked responses. The effects observed in individual experiments in the absence and in the presence of CGP 52432 are illustrated in the histograms of Figure 1*C* and in the trend plots of Figure 1*E*. In previous studies, $GABA_B$ receptor agonists did not cause detectable postsynaptic effects in MSNs, and paired-pulse stimulation experiments pointed to a presynaptic site of action (Calabresi et al., 1992; Nisenbaum et al., 1993). In our experiments, paired-pulse protocols would be difficult to interpret because, unlike the case of bath-applied agonists, $GABA_B$ receptors are activated transiently by GP stimuli. To test for possible postsynaptic contributions to the effects caused by $GABA_B$ receptor activation, in some experiments, we applied a 200 ms negative current step (10–40 pA) 200 ms after GP stimulation. The membrane potential displacement caused by these steps was measured at the end of the current injection, to minimize the effects of any residual depolarizations induced by the GP stimuli. As illustrated by the example of Figure 1*F*, in 6/6 experiments (in which significant $GABA_B$ receptor-mediated effects on CC-evoked responses were present), the input resistance was not significantly different when the step was preceded by GP stimuli (on average, 102 ± 2% of control).

We concluded that synchronous activation of MSNs caused inhibition of glutamatergic synapses onto MSNs, through GABA release leading to the activation of presynaptic $GABA_B$ receptors.

Presynaptic inhibition is maximal at 500 ms interval and disappears at 3 s

To determine the time course of the inhibition of glutamatergic responses caused by antidromic activation of MSNs, we performed experiments featuring two different time intervals between CC and GP stimulations. These experiments were similar to those described above but consisted of three repeated cycles, one without GP stimulation, one in which GP stimulation preceded CC stimulation by 500 ms and one in which GP stimulation preceded CC stimulation by either 1, 2, or 3 s. Due to the fact that repeated GP stimulation elicited long-tailed glutamatergic

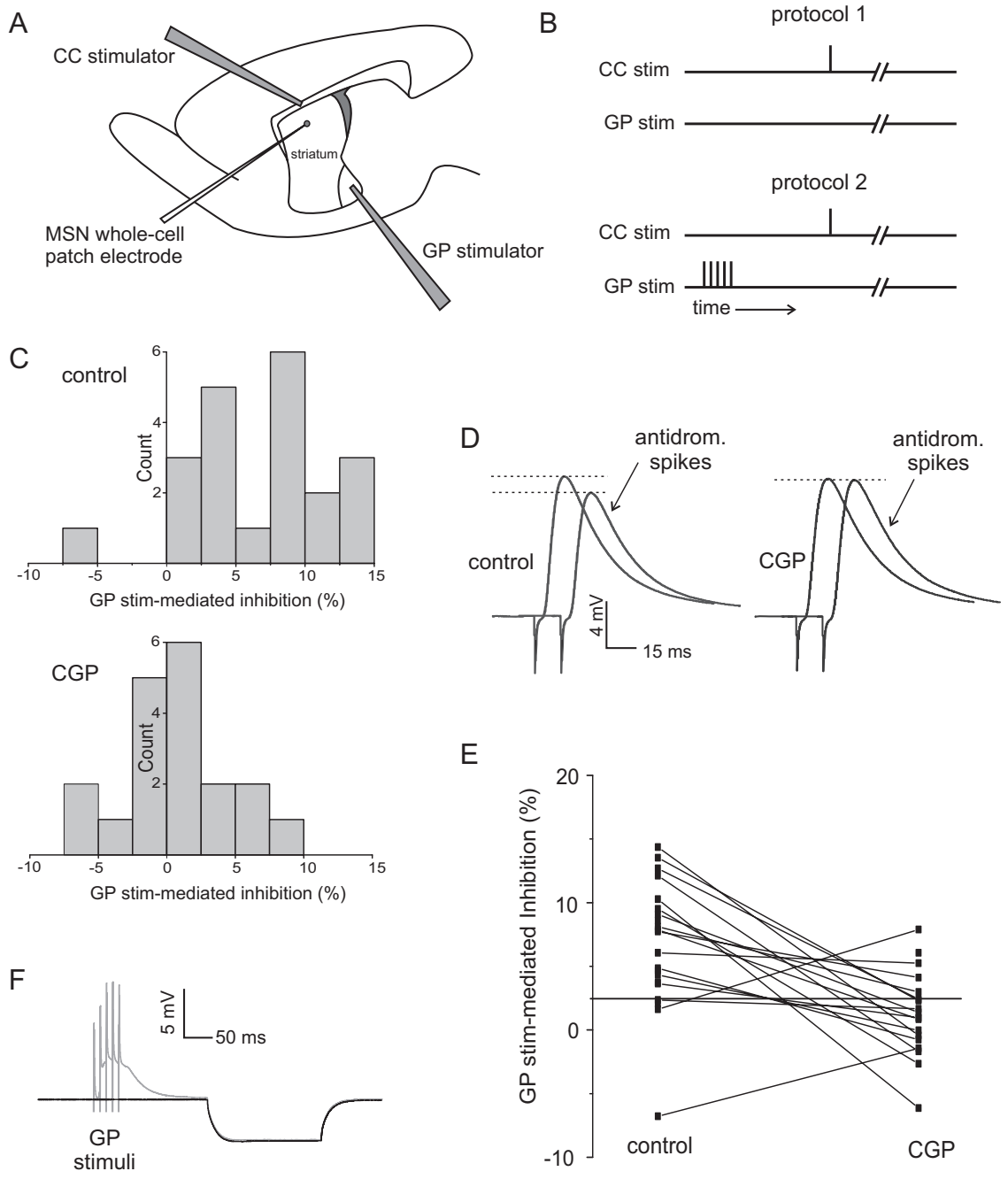

Figure 1. Antidromic activation of MSNs causes $GABA_B$ receptor-dependent inhibition of corticostriatal inputs. ***A***, Typical positioning of recording and stimulating electrodes. A MSN was recorded in the dorsolateral striatum. Corticostriatal fibers were activated by electrical stimulation delivered to the CC. MSN axons were antidromically activated by electrical stimulation delivered to the GP. ***B***, Stimulation sequence. Two consecutive stimulation protocols (each lasting 10 s) were continuously applied. In the first one, a single stimulation to the CC evoked control responses in the MSN. In the second protocol the CC stimulation was preceded (by 0.5 s) by a train of five stimuli in the GP, to elicit GABA release from a population of MSNs. This two-protocol cycle was applied without interruptions at least 75 times for each pharmacological condition. ***C***, Distribution of the average inhibitory effects of MSN antidromic activation on cortically evoked EPSPs observed in 21 experiments in control solution (top) and in the presence of CGP 52432 (bottom). ***D***, $GABA_B$ receptor-mediated inhibition in a representative experiment. Traces are averages of CC-evoked responses without or with preceding GP stimulation. In control solution, cortical inputs are inhibited by preceding antidromic activation of MSNs. In the presence of CGP 52432, these inhibitory effects are abolished. ***E***, Inhibitory effects of antidromic MSN stimulation on cortical responses in individual experiments. In each case, CGP 52432 was applied after the stimulation protocols had been performed in control solution. ***F***, A representative example of the lack of effects of GP stimuli on MSN input resistance. The trace in black is the average of 90 consecutive steps (200 ms, −30 pA) applied without preceding GP stimuli. The gray trace is the average of 90 consecutive steps (200 ms, −30 pA) applied 200 ms after the GP stimuli. Current steps were delivered every 10 s and GP stimuli preceded every other step.

responses in MSNs, it was not technically possible to test intervals smaller than 500 ms (Blomeley et al., 2009; Blomeley and Bracci, 2011). In all experiments, the inhibitory effects were larger for 500 ms intervals than for the other interval tested. In 14/14 of these experiments, significant ($p < 0.05$) inhibitory effects were observed at 500 ms intervals; at 1 s intervals, significant inhibition was observed in 4/4 experiments. At 2 s intervals, significant inhibition was observed in 2/4 experiments. At 3 s intervals, no significant

inhibition was observed in six experiments. The results observed in each experiment are illustrated in Figure 2*A*. In 12 of these experiments, CGP 52432 was subsequently added. In the presence of CGP5242, no significant inhibition was present for 500 ms intervals in 11/12 experiments and in 12/12 experiments for longer time intervals (1–3 s). A representative experiment in which two intervals were tested is illustrated in Figure 2*B*.

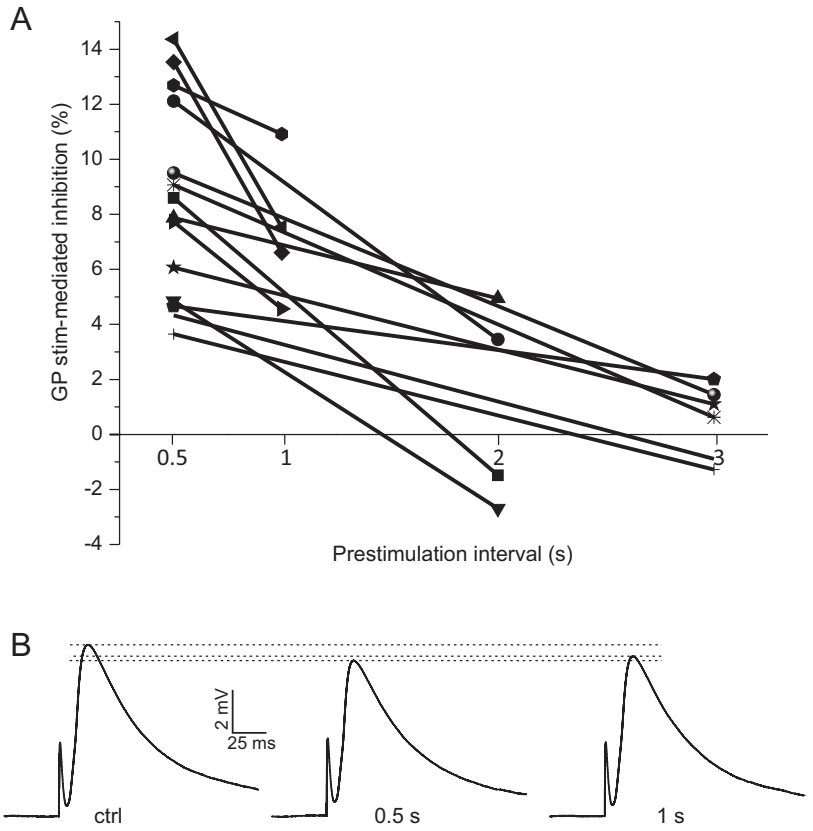

Figure 2. The inhibitory effects of MSN antidromic activation are maximal at 0.5 s interval. In each of these experiments, a second interval was tested in addition to 0.5 s. Thus, a cycle comprising three consecutive stimulation protocols (no GP stim; GP stim at 0.5 s interval; GP stim at 1, 2, or 3 s intervals) was applied at least 75 times. ***A***, Each line represents a single experiment. All experiments produced the largest inhibition at 0.5 s with declining inhibition for intervals up to 2 s. No significant inhibition was seen at 3 s intervals. ***B***, Averaged traces from a representative experiment. Significant inhibition is seen at 0.5 and 1 s intervals; inhibition is however significantly ($p < 0.05$) smaller at 1 s.

GABA released by single NGFIs but not other GABAergic neurons inhibits cortical glutamate release

In addition to MSNs, GABA is released by three well characterized types of interneurons in the striatum: LTSI, FSI, and NGFI. We therefore investigated whether individual GABAergic neurons were capable of suppressing cortically evoked glutamate release onto MSNs by activating $GABA_B$ receptors. This was accomplished with paired recording experiments, in which a MSN and another striatal GABAergic neuron (located <100 μm apart in the dorsolateral striatum) were recorded simultaneously (Fig. 3*A*). In these experiments, every other CC stimulation was preceded by five spikes evoked in the GABAergic neuron (see Materials and Methods). The interval between the first spike and the CC stimulus was 500 ms for all the experiments. The two consecutive stimulation protocols used are illustrated in Figure 3*B*. In each individual experiment out of a total of 57 MSN-MSN and 7 FSI-MSN paired recordings (performed in rats), spikes in a neighboring neuron (either another MSN or a FSI) failed to affect the CC-evoked responses of MSNs significantly (on average, responses preceded by spikes in another MSN were 101 $\pm$ 2% of control, whereas those preceded by spikes in a FSI were 99 $\pm$ 2% of control). We also performed 24 LTSI-MSN paired recordings using NPY-reporting BAC mice (Fig. 3*C*; see Materials and Methods for details). In each individual experiment, spikes in the LTSIs failed to significantly affect the CC-evoked responses of the simultaneously recorded MSNs (on average, responses preceded by spikes in an LTSI were 100 $\pm$ 1% of control). Examples of the absence of effects of spikes in a neighboring LTSI or an FSI on MSN responses are shown in Figure 3*D*.

We concluded that a burst of spikes in individual MSNs, FSIs, or LTSIs is not sufficient to elicit $GABA_B$ receptor-dependent inhibition of glutamatergic responses of a neighboring MSN. A novel striatal GABAergic interneuronal type, termed neurogliaform interneuron, has been recently described (Ibáñez-Sandoval et al., 2011). These interneurons also express NPY, have distinctive electrophysiological properties (Fig. 3*E*), and elicit large, long-lasting GABAergic IPSPs in MSNs (English et al., 2012). Therefore, we used paired recordings from NGFI-MSN pairs in BAC mice to test for their ability to cause $GABA_B$ receptor-mediated inhibition of glutamate responses.

In contrast to the other GABAergic neurons tested, we found that in 5/11 NGFI-MSN paired recording experiments, spikes in the NGFI significantly inhibited subsequent CC-evoked responses in MSNs ($p < 0.05$; Fig. 3*F*,*G*). On average, in these five cases, EPSP inhibition induced by NGFI spikes was 4.1 $\pm$ 0.3%.

In seven of these experiments, including all cases in which significant effects were observed in control solution, CGP 52432 was subsequently applied, abolishing any NGFI-mediated inhibition (Fig. 3*F*,*G*). We concluded that NGIs are the only GABAergic neurons in the striatum capable of individually triggering $GABA_B$ receptors-mediated inhibition of glutamate responses through a burst of spikes.

Discussion

The main finding of this study is that endogenous GABA, released by either a population of striatal MSNs or an individual NGFI, can depress glutamatergic inputs to MSNs by activating $GABA_B$ receptors. Conversely, individual MSNs, FSIs, or LTSIs did not elicit detectable $GABA_B$ receptor-mediated effects under the conditions of this study.

Our paired recording experiments clearly showed that a burst of five spikes in a single MSN was never able to produce significant $GABA_B$ receptor-mediated inhibition of glutamatergic inputs to a neighboring MSN. In contrast, such inhibition could be easily elicited by activating a population of MSNs with five antidromic stimuli delivered at the same frequency. This protocol excites similar numbers of striatopallidal and striatonigral MSNs, eliciting orthodromic spikes in their axon collaterals and synchronous release of GABA (López-Huerta et al., 2013). The most

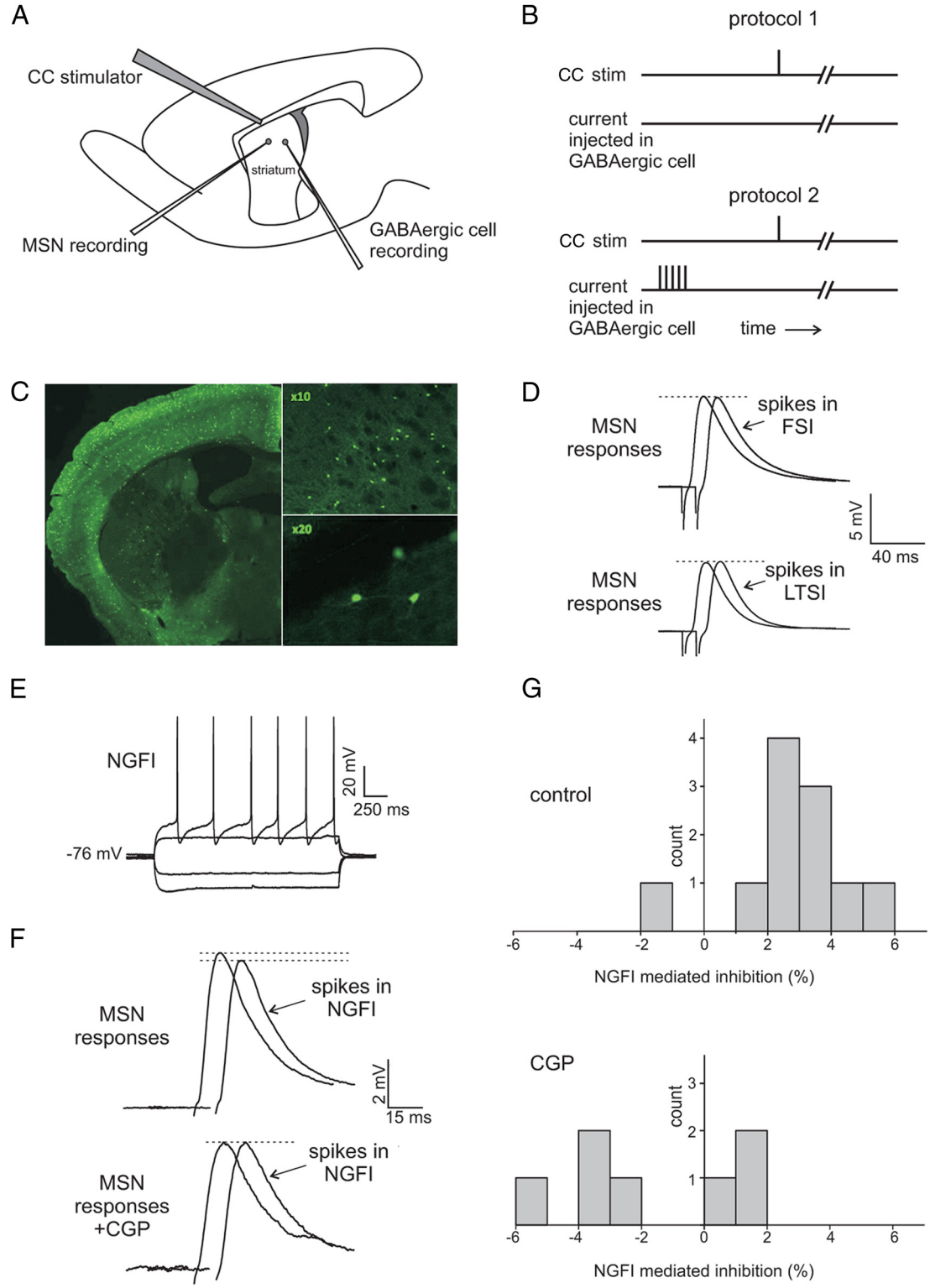

Figure 3. Individual NGFIs inhibit corticostriatal responses via $GABA_B$ receptors. ***A***, Positioning of recording and stimulating electrodes in dual recording experiments. A MSN and a second GABAergic neuron were recorded in the dorsolateral striatum. The distance between the two recorded cells was <100 μm. Corticostriatal fibers were activated by stimulation in the CC. ***B***, Stimulation sequence. Similar to Figure 1*B*, two stimulation protocols were applied consecutively. In the first protocol (10 s duration) a single CC stimulus was delivered. In the second protocol (bottom, 10 s duration), a single CC stimulation was preceded by five short (5 ms) depolarizing current pulses in the GABAergic cell, each of which elicited one action potential. This two-protocol cycle was applied without interruptions at least 75 times for each pharmacological condition. ***C***, Coronal slice image of GFP fluorescence from NPY-GFP-expressing BAC transgenic mice. In the striatum, GFP-expressing neurons are either LTSIs or NGFIs. ***D***, Two representative examples of the lack of effects of spikes of FSIs and LTSIs on the CC-evoked responses of neighboring MSNs. In the FSI-MSN experiment (top), each trace is the average of the MSN responses to a CC stimulus either preceded (right) or not preceded (left) by spikes in the FSI. In the LTSI-MSN experiment (bottom, different animal), each trace is the average of MSN responses either preceded (right) or not preceded (left) by spikes in the LTSI. ***E***, Typical electrophysiological properties of a NGFI revealed by negative and positive current pulses. Note large, slow spike-afterhyperpolarizations. ***F***, Average traces from a representative experiment. In control solution, MSN responses to CC stimulation were significantly ($p < 0.05$) inhibited by preceding NGFI action potentials. In CGP 52432, this inhibition was abolished. ***G***, Distribution of the inhibitory effect of NGFI action potentials on cortical responses of MSNs from 11 experiments in control solution (top), 7 of which lasted enough for subsequent application of CGP 52432 (bottom).

likely explanation for these results is therefore that a relatively large amount of GABA needs to be released to activate presynaptic $GABA_B$ receptors located on glutamatergic afferent. MSN-MSN GABAergic synapses tend to be formed on the dendritic shafts, whereas corticostriatal glutamatergic inputs are mainly formed on dendritic spines (Boyes and Bolam, 2007). Therefore, activation of presynaptic $GABA_B$ receptors located on glutamatergic terminals requires substantial diffusion of GABA in the extrasynaptic space. Apparently, this took place effectively only when a number of MSNs were synchronously activated. GP stimulation causes antidromic activation of both striatopallidal and striatonigral MSNs, but it does not allow precise identification of the size of the stimulated MSN population. A pallidostriatal GABAergic projection has been demonstrated, but it targets selectively striatal interneurons rather than MSNs (Bevan et al., 1998) and therefore it is unlikely to have played a role in the observed phenomenon.

Although we did not demonstrate directly that the present effects of $GABA_B$ receptors were presynaptic, previous experiments performed with exogenous agonists strongly suggest that this was the case. Indeed, application of $GABA_B$ receptor agonists has been shown to reduce glutamatergic EPSPs of MSNs through a presynaptic mechanisms, whereas no postsynaptic effects were observed (Calabresi et al., 1991; Nisenbaum et al., 1993). This is somehow puzzling, as $GABA_B$ receptors are found postsynaptically on MSNs (Lacey et al., 2005). Consistent with the previous electrophysiological experiments, we never observed $GABA_B$ receptor-mediated postsynaptic effects caused by spikes in MSN populations or individual NGFIs. Further studies will be required to reveal whether postsynaptic $GABA_B$ receptors are functionally impaired, or alternatively, mediate effects that are not detected by standard electrophysiological techniques.

In this study, glutamatergic responses were evoked by electrical stimulation of the portion of CC located between the cortex and the striatum. Although this procedure can be expected to produce preferential activation of corticostriatal fibers, it is likely that some thalamostriatal axons were also activated. Presynaptic $GABA_B$ receptors are found on both corticostriatal and thalamostriatal terminals (Lacey et al., 2005). Further studies will be required to establish whether specific features of $GABA_B$-mediated inhibition differ in the two sets of afferents.

The present results complement our previous findings that different populations of MSNs control the glutamatergic terminals in opposite ways either through activation of presynaptic NK1 receptors by substance P, or of presynaptic μ-opioid receptors by enkephalin (Blomeley et al., 2009; Blomeley and Bracci, 2011).

The time course of $GABA_B$ receptor-mediated inhibitory effects is similar to that observed for the activation of μ-opioid receptors (Blomeley and Bracci, 2011). In that case, inhibition of glutamate inputs was found to peak 500 ms after a burst of spike and to be still present, although reduced, after 1 s, and in some cases, after 2 s. This is slower than substance P-mediated facilitation, that was found to peak after 250 ms (Blomeley and Bracci, 2011). The different time course of facilitatory and inhibitory presynaptic interactions is likely to give rise to specific network dynamics that may be key to the striatal function.

An important difference between the previously studied presynaptic interactions and those mediated by $GABA_B$ receptors is that the former could be elicited by spikes in individual MSNs. In the case of GABA acting on $GABA_B$ receptors, the effects require synchronous activation of several MSNs. On the other hand, individual NGFIs, that do not express substance P or enkephalin, were capable of activating presynaptic $GABA_B$ receptors. These neurons, that were recently discovered, elicit large and long-lasting $GABA_A$ receptor-mediated IPSPs in MSNs (Ibáñez-Sandoval et al., 2011; English et al., 2012), consistent with a strong release of GABA from their terminals. The present results show that they also cause an even slower presynaptic inhibition of the excitatory inputs to MSNs. Although the effects caused by spikes in a single NGFI were relatively small, many such interneurons are in the position to affect the input to an MSN (Ibáñez-Sandoval et al., 2011); furthermore, the all-or-none nature of spike generation means that the ability of a glutamatergic input to drive an MSN above threshold may be impaired even by a small reduction in its amplitude. Thus, NGFIs are in a position to exert a strong influence on the local striatal circuits. It will be important from a functional point of view to determine the nature of the glutamatergic inputs that these interneurons receive from the cortex and the thalamus. Importantly, spikes in individual cholinergic interneurons also cause presynaptic inhibition of glutamatergic inputs to MSN (Pakhotin and Bracci, 2007; Ding et al., 2010).

Collectively, these observations provide a novel picture of the striatal network, in which rapid feedforward and feedback GABAergic inhibition through ionotropic $GABA_A$ receptors is accompanied by slower presynaptic metabotropic interactions mediated by peptides, GABA, and acetylcholine. It is tempting to speculate that these presynaptic interactions, whether facilitatory (substance P) or inhibitory (enkephalin, acetylcholine, and GABA), will create a grid of primed or suppressed synapses after an initial barrage of cortical inputs. This may be an effective way to create dynamic cell assemblies, particularly prone to be excited by further cortical inputs, whereas other groups of projection neurons are denied access to cortical excitation. Computational models will be useful to explore how these presynaptic interactions affect action selection and reinforcement learning in the striatum.

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