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Autonomous Subthalamic Nucleus Activity in a Mouse Model of Parkinson’s Disease: Mechanisms of Disruption and Consequences of Its Restoration

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Abstract

Parkinson’s disease (PD) is a movement disorder that arises following the degeneration of dopamine-producing neurons of the substantia nigra pars compacta (SNC). Patients experience a host of limiting motor symptoms such as rigidity, tremor, and a paucity of controlled movement generation. These symptoms often correspond with a predominance of abnormally synchronous activity among neurons in the cortico-basal ganglia-thalamo-cortical loop, which moderates limbic, associative, and motor processes. This thesis focuses on the electrophysiological activity of one basal ganglia component, the subthalamic nucleus (STN), and how it changes in mouse models of PD, with the aim of targeting its pathological behavior for therapeutic intervention. Herein, a combination of ex vivo electrophysiology, 2-photon imaging, optogenetics, chemogenetics, immunohistochemistry, and behavioral assays were employed to elucidate the circuit triggers, cellular mechanisms, and therapeutic targeting potential of aberrant STN activity.

The STN is situated at an intersection of excitatory and inhibitory inputs where it integrates incoming signals from the cortico-basal ganglia-thalamo-cortical loop and excites the circuit’s output structures. This synaptic integration is influenced by the timing of autonomously-generated action potentials that are produced rhythmically and continuously by STN neurons at rates typically between 5 and 20 Hz in the mouse, thus rendering the influence of synaptic input dependent on the neurons’ recent intrinsic firing history. Several models of experimental PD exhibit a complete loss or a severe reduction in the frequency and regularity of autonomous firing in STN neurons during ex vivo electrophysiological recordings, including the acute 6-hydroxydopamine (6-OHDA) toxin lesion and the progressive genetic MitoPark mouse models tested in this study. While dopaminergic input from the SNC can modulate STN activity, its loss is not the cause of the parkinsonian firing phenotype because in dopamine-intact mice, chronic
chemogenetic activation of D2 dopamine receptor-expressing striatal projection neurons (D2-SPNs, whose output is elevated in PD) is sufficient to reproduce the STN firing perturbation of the 6-OHDA model (in which the effect is occluded). The result of augmented D2-SPN inhibition of the inhibitory external segment of the globus pallidus (GPe) is in turn a disinhibition of the downstream STN. Periodic GPe-STN inhibition is phase-offset with excitatory cortical excitation in the synchronously-oscillating parkinsonian brain, which generates greater activation of NMDA receptors (Rs) in STN neurons and elevated calcium influx through them. Indeed, autonomous STN activity was disrupted in dopamine-intact mice by *ex vivo* activation of NMDARs for 1 hr, but this effect was occluded in PD mice. Furthermore, knockdown of STN-NMDARs in PD mice prevented the loss of rhythmic autonomous firing after dopamine depletion. Altogether these results indicate that 1) elevated D2-SPN output that disinhibits the STN is responsible for triggering the firing perturbation in PD mice, and 2) STN NMDAR activation is both necessary and sufficient to produce the downregulation of intrinsic activity in PD mice.

Maintaining a hyperpolarized membrane potential is a metabolically costly endeavor, particularly in an autonomously firing and/or prolifically stimulated neuron. With additional Ca\(^{2+}\) influx through NMDARs comes the production of reactive oxygen species (ROS) in mitochondria that can engage intracellular signaling pathways and trigger metabolic adaptations to the neuron’s electrochemical environment. In line with this theory, mitochondrial oxidation in STN neurons was elevated in 6-OHDA-treated mice, and acute breakdown of the ROS H\(_2\)O\(_2\) *ex vivo* rescued autonomous firing, implying ROS generation in the STN may be translating NMDAR activation into a suppression of neuronal excitability. STN NMDAR stimulation has been shown to activate the ATP-sensitive K\(^+\) (K\(_{\text{ATP}}\)) channel, which hyperpolarizes the membrane and reduces intrinsic firing. H\(_2\)O\(_2\) has also been shown to activate these channels, and indeed blocking K\(_{\text{ATP}}\) channels *ex vivo* restored intrinsic firing and occluded the rescue by H\(_2\)O\(_2\) breakdown. These results argue
that elevated STN NMDAR activation and Ca\(^{2+}\) influx leads to increased production of ROS and H\(_2\)O\(_2\)-mediated activation of the K\(_{\text{ATP}}\) channel, which downregulates autonomous activity.

Finally, STN neurons were chemogenetically activated to restore intrinsic excitability in PD mice. *Ex vivo*, chemogenetic STN activation in 6-OHDA-lesioned mice diminished the synaptic drive from optogenetically stimulated motor cortical inputs to the STN, an indication that normalized autonomous firing reduces the STN’s propagation of incoming synchronous oscillatory activity. As abnormally synchronous activity corresponds with symptoms of PD, its reduction by restoring autonomous STN activity was predicted to ameliorate motor deficits in these mice. Indeed, chemogenetic activation of the STN reduced forelimb use asymmetry in the unilateral 6-OHDA mouse model. Thus, despite appearances that downregulation of STN activity in response to elevated NMDAR activation would be adaptive to resist excitotoxicity, an individual neuron’s response to its immediate environment (e.g., energy availability and synaptic input) cannot take into account the entire circuit’s interactions and output. This thesis argues the STN’s reduction of intrinsic firing is ultimately maladaptive in the context of the parkinsonian brain, and its correction is therapeutic. This study therefore contributes to the PD field’s understanding of pathophysiology in the STN following dopamine loss and suggests a novel therapeutic intervention to treat motor symptoms of the disease.
Acknowledgements

First and foremost, I would like to thank my adviser, Dr. Mark Bevan, for his guidance and support during the 6 years I spent under his mentorship. Always generous with his time and eager to share his expertise and enthusiasm for neuroscience, Mark sets among the highest standards for the quality of science done in the basal ganglia, and I am proud to have been a member of his lab. In addition to my committee and the NUIN leadership, I would also like to thank Drs. Jeremy Atherton and Hongyuan Chu, who always made themselves available to help troubleshoot technical problems or answer questions—I could not have asked for better examples of independent scientists to learn from and emulate. I certainly would not have made it this far without the friendship of all the members of the Bevan lab during my tenure there, and for that I am deeply appreciative.

My family has always supported me in whatever I do, and my time in graduate school has been no exception. Their achievements inspire me, and their love sustains me. Finally, I would like to thank my partner Brian. He knows why.
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
</tr>
<tr>
<td>AAV</td>
<td>adeno-associated virus</td>
</tr>
<tr>
<td>AC</td>
<td>adenylyl cyclase</td>
</tr>
<tr>
<td>ACSF</td>
<td>artificial cerebrospinal fluid</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazole</td>
</tr>
<tr>
<td>AP</td>
<td>action potential</td>
</tr>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
</tr>
<tr>
<td>BG</td>
<td>basal ganglia</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CNO</td>
<td>clozapine-n-oxide (DREADD activator)</td>
</tr>
<tr>
<td>D1-SPN</td>
<td>type 1 dopamine receptor-expressing striatal projection neuron</td>
</tr>
<tr>
<td>D2-SPN</td>
<td>type 2 dopamine receptor-expressing striatal projection neuron</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DAT</td>
<td>dopamine transporter</td>
</tr>
<tr>
<td>DREADD</td>
<td>designer receptor exclusively activated by designer drugs</td>
</tr>
<tr>
<td>EPSC</td>
<td>excitatory post-synaptic current</td>
</tr>
<tr>
<td>EPSP</td>
<td>excitatory post-synaptic potential</td>
</tr>
<tr>
<td>ETC</td>
<td>electron transport chain</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-Aminobutyric acid</td>
</tr>
<tr>
<td>GPCR</td>
<td>G protein-coupled receptor</td>
</tr>
<tr>
<td>GPe</td>
<td>globus pallidus pars externa</td>
</tr>
<tr>
<td>GPi</td>
<td>globus pallidus pars interna</td>
</tr>
<tr>
<td>HD</td>
<td>Huntington’s disease</td>
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</tbody>
</table>
hM3Dq – Gq-linked human M3 muscarinic receptor DREADD
hM4Di – Gi-linked human M4 muscarinic receptor DREADD
IP – intraperitoneal
K<sub>ATP</sub> – ATP-sensitive potassium channel
Kir – inwardly-rectifying potassium channel
L-DOPA – levodopa
LID – levodopa-induced dyskinesia
LTD – long-term depression
LTP – long-term potentiation
M1 – primary motor cortex
MFB – medial forebrain bundle
MPTP – 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
Nav – voltage-gated sodium channel
NET – norepinephrine transporter
NMDA – N-methyl-D-aspartate
PD – Parkinson’s disease
PKA – protein kinase A
PPN – pedunculopontine nucleus
PSTN – parasubthalamic nucleus
PV – parvalbumin
R – receptor (e.g. NMDAR)
RASSL – receptor activated solely by synthetic ligands
rM3Ds – Gs-linked M3 muscarinic receptor DREADD
RNS – reactive nitrogen species
ROS – reactive oxygen species
SC – subcutaneous
SIF – synthetic interstitial fluid
SK – small-conductance calcium-activated potassium channel
SNC – substantia nigra pars compacta
SNR – substantia nigra pars retiulata
STN – subthalamic nucleus
TFAM – mitochondrial transcription factor A
TH – tyrosine hydroxylase
TTX – tetrodotoxin
ZI – zona incerta
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CHAPTER 1: INTRODUCTION

Humans are exceptionally capable of learning and making precise, coordinated movements—from serving a tennis ball to playing a piano concerto. Vital to the execution of these movements is the basal ganglia, a collection of interconnected deep brain structures that are integral to the refinement, selection, and execution of motor commands, as well as associative and limbic functions. While the latter two are imperative for cognition and emotion (Obeso et al 2014), this dissertation will focus on the basal ganglia’s role in motor function and dysfunction, particularly the subthalamic nucleus (STN) in the context of Parkinson’s disease (PD).

1.1: The STN and the basal ganglia

*Basal ganglia components and connectivity*

The basal ganglia comprise a series of interconnected deep brain structures: the caudate, putamen, nucleus accumbens, and olfactory tubercle (referred to collectively as the striatum due to their striated appearance); the globus pallidus (segregated into external and internal segments, GPe and GPi); the substantia nigra (containing the pars reticulata and pars compacta, SNr and SNc); and the STN. With the exception of the SNc and STN, the main projection neurons of each basal ganglia nucleus are inhibitory and release the neurotransmitter gamma-Aminobutyric acid (GABA). Neurons of the SNc release the neuromodulator dopamine (DA), which has different effects on its targets depending on their receptor complement. Finally, the STN is excitatory and releases the neurotransmitter glutamate (Glu). These nuclei of the basal ganglia are each topographically organized in sub-regions based on their participation in motor, limbic, and associative loops (reviewed in Parent and Hazrati 1995). The motor territory occupies the
dorsolateral aspect of the STN, represents somatotopic maps within the sub-region, and will be the area of focus for this thesis (Afsharpour 1985b; Nambu et al., 1996; Kita et al., 2014).

The basal ganglia receive extensive glutamatergic input from the cortex via the striatum and the STN, and their output converges in the GPi/SNr complex. Traditionally, information flow through the basal ganglia has been described for three main pathways: the direct, indirect, and hyperdirect pathways (Figure 1.1.1). The canonical direct pathway designates the circuit from D1 dopamine receptor-expressing striatal projection neurons (D1-SPNs) directly to the basal ganglia output nuclei, thereby disinhibiting thalamic, cortical, and brainstem regions to allow selected action execution. The indirect pathway is mediated by D2-SPN transmission to basal ganglia output nuclei via the reciprocally-connected GPe-STN network (Albin et al., 1989). The hyperdirect pathway proceeds through projections from the cortex directly to the STN, which then excites inhibitory output nuclei. However, emerging evidence suggests that the “movement-promoting” function of the direct pathway, and “movement-inhibiting” designation of the indirect pathway is overly simplistic. For example, Rui Costa’s group used a fiber optic implanted in the striatum of mice recording the real-time fluorescence of genetically encoded calcium indicators to observe concurrent activation of D1- and D2-SPNs in vivo during action initiation (Cui et al., 2013). They further argued that coordinated activity of the direct and indirect pathways is required for movement initiation, as optogenetic manipulations of either D1- or D2-SPNs prior to action sequence commencement only delays its start (Tecuapetla et al., 2016). However these findings are not completely incompatible with the classical movement-promoting and –inhibiting functions of the direct and indirect pathways, respectively, as concurrent excitation of intended actions and inhibition of everything else would comply with both theories. Further discussion of the field’s current understanding of functional processing in the basal ganglia in the context of PD is discussed in Section 1.2.
Figure 1.1.1: Cortico-basal ganglia-thalamo-cortical circuit connectivity. The direct pathway of information flow through the basal ganglia begins with cortico-striatal input onto D1-SPNs, which directly inhibit output nuclei (GPi/SNr). The indirect pathway receives cortical input onto D2-SPNs, which inhibit GPe neurons. The GPe is reciprocally connected with the glutamatergic STN, which excites the GPi/SNr. Finally, the hyperdirect pathway describes the direct cortical excitation of the STN, thereby bypassing striatal circuitry and quickly exciting output nuclei. The STN also receives glutamatergic input from thalamus, the superior colliculus, and the pedunculopontine nucleus (PPN; discussed further in Section 4.2).
**STN output and its functional role**

As the main excitatory influence on basal ganglia output, and situated directly upstream of the GPi/SNr in the indirect and hyperdirect pathways, the STN is positioned to be an important integrator of circuit activity in the cortico-basal ganglia-thalamo-cortical loop. The morphology of STN neurons itself suggests their functional capabilities. Their relatively far-reaching, ellipsoidal dendritic arbors allow them to integrate synaptic input from a broad range of sources in the topographically organized nucleus (Yelnik and Percheron 1979). Furthermore, the dendritic spines adorning STN dendrites (Chan et al., 1983) may play an important role in a cell’s response to glutamatergic inputs, both in terms of the integration of electrical signals (e.g. linear summing, Araya et al., 2006a) that determines its output as well as cellular and synaptic plasticity that can alter future responses (reviewed in Araya 2014).

This integrated signal is then passed on to the output nuclei of the basal ganglia. STN-GPi projections observed in the spider monkey (Hazrati and Parent 1992) showed dense axon collaterals surrounding somata and proximal dendrites in a wide distribution of pallidal neurons, suggesting a broad and potent influence of the STN on the GPi. Recordings of GPi output neurons in rats by Kita (1992) during cortical stimulation showed a short-latency excitatory post-synaptic potential (EPSP) followed by an inhibitory PSP (IPSP) and a second, late EPSP (also demonstrated by Nambu et al., 2000; Chiken and Nambu 2013). Lesioning the STN eliminated the EPSPs while preserving the IPSPs, consistent with a hyperdirect and indirect pathway input to GPi via the STN (with the IPSPs putatively arising from striatal neurons of the direct pathway; Figure 1.1.1). The short-latency disynaptic GPi EPSP via cortico-STN projections is considerably faster than the disynaptic cortico-striatal circuit (Kita 1992; Nambu et al., 2000). Thus the hyperdirect pathway from the cortex to the STN is thought to quickly relay excitation to basal ganglia output nuclei, thereby transmitting a short-circuit “stop” signal to suppress unwanted
motor, limbic, and associative output (Maurice et al. 1999; Nambu et al., 2002; Tachibana et al. 2008). In monkeys, GPi ablation has its most profound effects on the suppression of unwanted, antagonist muscle activity (Horak and Anderson 1984; Mink and Thach 1991). Mink and Thach (1993) therefore proposed a “center-surround” or “focused selection” model of basal ganglia circuit function to describe how GPi activity encodes specific outputs, such that corollary signals from the cortex excite a particular subset of neurons in the STN that activate the appropriate GPi units to suppress unwanted/antagonistic programs. Meanwhile, the same signal selectively inhibits GPi via direct pathway striato-pallidal inputs to relieve the appropriate thalamic/brainstem areas from inhibition. Further refinement of the center-surround signal is achieved by lateral inhibition within the striatum (Mink and Thach, 1993). This role for STN is supported by outcomes in Wichmann et al. (1994) showing STN units respond at a latency following movement that suggest they are not involved in its initiation, but rather its inhibitory modification, as firing increases predominate over decreases after movement onset. In addition, microstimulation of STN cells associated with movement do not themselves elicit movements (Wichmann et al., 1994). The STN therefore plays an important role in the focused selection and refinement of motor output by virtue of its position in the indirect and hyperdirect pathways upstream of basal ganglia output nuclei.

**STN autonomous activity and synaptic integration**

Like all the extra-striatal basal ganglia nuclei, the STN fires action potentials (APs) spontaneously in the absence of synaptic input (Bevan and Wilson 1999). An abundance of voltage-gated sodium (Nav) channels in the STN’s axosomatic compartment conduct a persistent and resurgent Na+ current that underlies spontaneous APs (Do and Bean, 2003), which are TTX- and hyperpolarization-sensitive (Bevan and Wilson 1999; Atherton et al. 2008). Subsequent Ca2+ influx through high-voltage activated Ca2+ channels engages a hyperpolarizing Ca2+-activated K+ current.
current (SK) that determines the frequency and precision of spontaneous APs (Hallworth et al., 2003; Bevan and Wilson 1999). Additionally, CaV1.2-1.3 and CaV3 channels, which are not active during autonomous firing, deinactivate with hyperpolarization and underlie the rebound burst firing seen in STN neurons following GABAergic inhibition (Bevan et al., 2002; Hallworth et al., 2003; Hallworth and Bevan, 2005). Strong stimulation of the GPe can inhibit STN neurons sufficiently to deinactivate NaV (and CaV) channels to reset or synchronize activity (Bevan et al., 2002; Hallworth and Bevan, 2005).

STN neurons receive potent yet sparse topographically organized GABAA-mediated inputs from the GPe, such that each GPe neuron innervates ~2% of the STN, and each STN neuron receives input from ~2% of the GPe (Baufreton et al 2009). The STN also receives glutamatergic input from the thalamus and every cortical lobe—parietal, occipital, temporal, frontal (Israel and Bergman, 2008)—mediated by Ca2+-permeable AMPA and NMDARs (Götz et al., 1997). The effect that these inputs have on STN neurons depends on the firing history immediately prior to the EPSP, which can be shaped by autonomous STN activity and inhibitory synaptic inputs that shift the threshold for spiking and excitability (through NaV deinactivation) of the neurons (Baufreton et al., 2005; Farries et al., 2010). Thus, the intrinsic membrane properties conferring autonomous firing and rebound burst activity to STN neurons render their firing output bi-directionally and temporally sensitive to input patterns, and make synaptic integration dependent on the phase of autonomous action potential firing (Wilson and Bevan 2011; Wilson 2013). As such, the autonomous activity of STN neurons likely serves an important function when integrating movement-selecting synaptic inputs.
Dopaminergic modulation of the basal ganglia

The basal ganglia are widely innervated by dopaminergic projections from the SNc, which modulate excitability and synaptic transmission in a spatially and temporally discrete manner. Dopamine release by nigral neurons encodes reward and salience cues, and imparts the capability to calculate reward prediction errors and induce activity changes and plasticity accordingly (reviewed by Schultz 2010). The striatum receives the densest dopamine innervation, where D1- or D2-type receptors (Rs) expressed predominantly on direct and indirect pathway SPNs, respectively, carry out the modulatory effects of DA (Bolam et al 2000; Gerfen et al 1990). D1Rs are G protein-coupled receptors (GPCRs) that couple to $G_s$ and $G_{olf}$ alpha subunits, which stimulate the production of cyclic adenosine monophosphate (cAMP) via adenylyl cyclase (AC) and thereby elevates protein kinase A (PKA) activation, which can then phosphorylate myriad targets. D1R and downstream PKA activation in D1-SPNs decreases the constitutive inwardly-rectifying K$^+$ (Kir) currents that keep the neurons in the hyperpolarized “down-state” and increases L-type Ca$^{2+}$ currents, leading neurons to be more excitable by their glutamatergic inputs (Gerfen and Surmeier 2011). Conversely, DA signaling in D2-SPNs does the exact opposite through $G_{i/o}$ coupling to a reduction in AC activation, leading to diminished Ca$^{2+}$ and Na$^+$ currents and elevated Kir channel activity that shunts glutamatergic excitation and resists transitions to the depolarized “up-state” (Gerfen and Surmeier 2011).

There is also evidence that DA modulates STN activity and synaptic transmission. Indeed, Hassani et al. (1997) showed dopaminergic projections to the STN in rats, and François et al. (2000) confirmed the observation in humans and monkeys. Furthermore, dopamine receptor activation in the rodent STN has been shown to increase intrinsic firing in slices (Cragg et al 2004; Zhu et al., 2002a; Wilson et al., 2006; Ramanathan et al., 2008; Loucif et al., 2008) and when iontophoretically applied in vivo (Mintz et al., 1986). STN D2R activation reduces Ca$_{v2.2}$ channel
current (which normally stimulates small conductance calcium-activated potassium (SK)
current), thereby increasing the excitability of the cell (Ramanathan et al., 2008). D1 receptors
activate the AC-cAMP pathway, which further increases autonomous STN activity via a cyclic
nucleotide-gated (CNG) cation channel and/or activation of Cav1 channels (Loucif et al 2008;
Baufreton et al 2003). Synaptic transmission is also modulated by dopamine in the STN, as Shen
and Johnson (2000) showed a presynaptic D2R-mediated decrease in EPSC and IPSC amplitudes
by reducing initial release probabilities (each likely through different mechanisms, per Nicola and
Malenka’s 1997 work in the nucleus accumbens). Thus, DA is a potent modulator of the basal
ganglia, and it is therefore not surprising that its loss produces dramatic changes in cortico-basal
ganglia-thalamo-cortical circuit activity and severe behavioral deficits, as happens in PD.

1.2: Parkinson’s disease

James Parkinson first described the “shaking palsy” in 1817. Today, PD affects over 10 million
people worldwide, and the likelihood of developing it increases dramatically after age 60. It is
cased by the degeneration of dopamine neurons in the SNc, and patients typically present with
symptoms after 60% of SNc neurons have died (Dauer and Przedborski, 2003). Dauer and
Przedborski (2003) succinctly review numerous common symptoms of Parkinsonism and their
profound daily impact on quality of life:

PD tremor occurs at rest but decreases with voluntary movement, so typically does not
impair activities of daily living. Rigidity refers to the increased resistance (stiffness) to
passive movement of a patient’s limbs. Bradykinesia (slowness of
movement), hypokinesia (reduction in movement amplitude), and akinesia (absence of
normal unconscious movements, such as arm swing in walking) manifest as a variety of
symptoms, including paucity of normal facial expression (hypomimia), decreased voice
volume (hypophonia), drooling (failure to swallow without thinking about it), decreased
size (micrographia) and speed of handwriting, and decreased stride length during walking.
Bradykinesia may significantly impair the quality of life because it takes much longer to
perform everyday tasks such as dressing or eating. PD patients also typically develop a
stooped posture and may lose normal postural reflexes, leading to falls and, sometimes,
confinement to a wheelchair. Freezing, the inability to begin a voluntary movement such
as walking (i.e., patients remain “stuck” to the ground as they attempt to begin moving), is a common symptom of parkinsonism. Abnormalities of affect and cognition also occur frequently; patients may become passive or withdrawn, with lack of initiative; they may sit quietly unless encouraged to participate in activities. Responses to questions are delayed, and cognitive processes are slowed (“bradyphrenia”). Depression is common, and dementia is significantly more frequent in PD, especially in older patients.

The triggers of DA neuron degeneration remain unresolved, the disease reduces life expectancy, and there is no cure. In the meantime, developing an understanding of intrinsic properties and circuit function at the basic science level is helping inform treatment development that drastically improves the quality of life for sufferers and their families.

**Striatal effects of DA depletion**

When the levels of DA diminish in the striatum, D1-SPNs consequently become less excitable and D2-SPNs more excitable, by virtue of their $G_s/G_{olf}$ and $G_{i/o}$-coupled endocellular signaling, respectively (Mitchell et al., 1989). On top of that, Shen et al (2008) demonstrated that at glutamatergic synapses, long-term depression (LTD) predominates in D1-SPNs and long-term potentiation (LTP) occurs preferentially in D2-SPNs in dopamine-depleted mice (supported by Thiele et al., 2014). This further biases the balance of direct and indirect pathways toward the latter when DA is scarce. Indeed, several studies report hyperactivity of the indirect pathway relative to the direct pathway in animal models of PD (e.g. Filion and Tremblay 1991; Mallet et al., 2006). The elevation of indirect pathway striato-pallidal D2-SPN activity leads to abnormal inhibition of GPe neurons, thereby disinhibiting the STN (Magill et al., 2001; Mallet et al., 2006, 2008b; Kita and Kita 2011). In support of this, Vila et al. (2000) demonstrated in rats that STN activity, as measured by metabolic and in vivo electrophysiological approaches, became both elevated and more irregular as dopaminergic neurons (per immunohistochemical labeling of the dopamine transporter, DAT) degenerated in the two weeks following 6-OHDA injection. In conjunction with diminished inhibitory input from striato-nigral D1-SPNs (e.g. Mallet et al.,
2006), these activity changes cause elevated excitation of the GPi/SNr and subsequent inhibition of output structures (illustrated in Figure 1.2.1).
Figure 1.2.1 Modeling Parkinsonism. In the absence of dopamine, D2-SPN inhibition of the GPe is elevated and D1-SPN inhibition of the GPi is reduced in this simple model of cortico-basal ganglia-thalamo-cortical circuit connectivity. This results in altered inhibition of the STN by GPe neurons, and may contribute to pathological plasticity (discussed in Section 4.1).
**Rate vs pattern models of the basal ganglia in PD**

According to the classical model put forward by Albin et al. (1989), this imbalance would explain the akinetic symptoms of PD by arguing the prominence of the indirect pathway over the direct overwhelms basal ganglia motor output targets with inhibition from the GPi/SNr and prevents movement (Bergman et al., 1990; Obeso et al. 2000b; Bateup et al. 2010). A study by Kravitz et al. (2010) that used selective optogenetic stimulation of either D1- or D2-SPNs showed that D2-SPN stimulation induced freezing and bradykinesia in control mice, and D1-SPN stimulation in Parkinsonian mice reduced motor deficits. Notably, however, many of these studies in experimental PD demonstrating changes in basal ganglia firing rates also report a shift in neuronal discharge patterns from predominantly tonic firing to persistent low-frequency oscillatory burst activity (Filion 1979; Filion and Tremblay, 1991; Hollerman and Grace, 1992; Bergman et al., 1994, 1998; Vila et al., 2000).

In fact, a burgeoning collection of literature shows that the pattern of firing and correlation with other brain areas, rather than overall activity level in neurons of the basal ganglia enable movement initiation and choice (e.g. Wichmann et al., 1994; Bevan et al., 2002; Mallet et al., 2008a; Engle and Fries 2010; Muthukumaraswamy 2010; Rosin et al., 2011). In idiopathic and experimental PD, subjects display abnormally exaggerated and correlated beta band (13-30 Hz) oscillations in the cortico-basal ganglia-thalamo-cortical circuit connected to impaired motor performance (Gatev et al., 2006; Gale et al., 2008; Kühn et al., 2009; Bronte-Stewart et al., 2009). Using transcranial alternating current stimulation (TACS), Jouidi and colleagues (2012) demonstrated that driving oscillatory activity in the cortex at 20 Hz reduced force generation in a go/no-go task in healthy humans, an impairment reflective of the Parkinsonian state. This corresponds to the reported prominence in healthy subjects of normal beta-frequency oscillations during tonic contraction, and the abatement of these oscillations when preparing for and
sustaining voluntary movement—called an event related desynchronization (ERD; Engle and Fries 2010; Pfurtscheller and Lopes da Silva 1999). If beta band activity in the healthy brain correlates with continuous (e.g. postural) muscle contractions and inversely correlates with movement onset, an abnormal persistence of beta oscillations like in PD would predict akinesia and rigidity. Indeed, recent experiments have shown that amelioration of motor symptoms of PD is closely associated with a reduction in persistently synchronous, highly correlated activity that dominates the Parkinsonian brain (Benabid et al., 2009; McConnell et al., 2012; Zaidel et al., 2010; Eusebio et al., 2012; Whitmer et al., 2012; Agnesi et al., 2013). It follows logically that a circuit oscillating uniformly and coherently can process and transmit very little—if any—unique information, and would be unable to refine and select movements as a functional basal ganglia does.

**Therapeutic approaches to PD**

The “gold standard” of PD therapeutics since the 1960s is oral DA replacement therapy, wherein the precursor levodopa (L-DOPA) is prescribed at a dose tempered by the patient’s response. In addition to restoring dopaminergic tone to the brain, L-DOPA may also provide neuroprotection by acting as an antioxidant (Camp et al. 2000). Most pharmacological PD treatments developed since L-DOPA work by augmenting or prolonging the effectiveness of the precursor (LeWitt 2009). Many patients of dopamine replacement therapy enjoy significant relief from motor dysfunction, but after 9 years of L-DOPA treatment, 9 in 10 patients experience dyskinesia (thus termed levodopa-induced dyskinesia or LID), which often involves uncontrolled hyperkinetic choreiform (forceful dance-like) movements that can be debilitating (Francardo and Cenci 2014). Bateup et al (2010) showed that D1-SPNs are required for LID to manifest in a mouse model, in support of the hypothesis that D1R-specific supersensitization is involved in its development.
(Cenci and Konradi 2010). Thus, L-DOPA treatment, while very effective for many, loses its therapeutic benefits in most patients over time.

When L-DOPA therapy becomes insufficient to manage Parkinsonian symptoms, doctors have turned to treatments that directly modify activity in basal ganglia circuit elements—namely by ablating targets or stimulating them electrically. Stereotaxic neurosurgery to eliminate the GPi ("pallidotomy") or the STN ("subthalamotomy") from the Parkinsonian brain circuitry yields an improvement in both PD symptoms and those of LID, remarkably without any apparent impairment to voluntary movement (Bergman et al, 1990; Laitinen 1995; Marsden and Obeso 1994). Pharmacological silencing of the STN and GPi by microinfusion of GABAR agonists and GluR antagonists has likewise been demonstrated to improve motor symptoms of experimental PD (e.g. Levy et al, 2001). Furthermore, Tachibana et al (2011) showed in MPTP monkeys that pharmacologically inhibiting the STN reduced pathological oscillations (8-15 Hz) in the STN and GPi and corresponded with an amelioration of motor symptoms, once again demonstrating an association between the reduction of coherent oscillations and therapeutic benefit.

Benabid et al.’s 1987 discovery that high frequency stimulation (HFS) of the thalamus is as effective as ablation thereof for the treatment of tremor motivated the investigation of this more adjustable and reversible neurosurgical method for targeting circuit abnormalities to treat PD. High-frequency deep brain stimulation (DBS) of the STN or GPi is currently employed in eligible patients to correct the cardinal symptoms of PD (Wichmann and Delong, 2006). While this intervention is at odds with the rate model proposed in Albin et al., (1989), in which increasing STN or GPi activity would yield Parkinsonian symptoms, direct electrical stimulation in these regions frequently corresponds with an immediate and profound improvement in motor control. Like the aforementioned STN silencing studies, therapy corresponds with a reduction in coherent
oscillations achieved by (presumably) driving activity in these subsets of neurons—a result that was inaccessible before DBS justified the use of implanted electrodes to both stimulate and record neural activity in human PD patients (Benabid et al., 2009; Bronte-Stewart et al., 2009; Eusebio et al., 2012; Whitmer et al., 2012; Agnesi et al., 2013; Trager et al., 2016). Interestingly, studies have also shown that L-DOPA similarly effects an abatement of widespread coherent oscillations, further supporting the correspondence between abnormally synchronous activity and motor symptoms (Gatev et al., 2006; Kühn et al., 2009; López-Azcárate et al., 2010). And the fact that patient outcomes can be improved by optimizing stimulation settings with temporally non-regular programs also speaks to the importance of firing patterns in the diseased basal ganglia (Farris and Giroux, 2013; Hess et al., 2013; Brocker et al., 2013). Indeed, closed-loop DBS systems—which actively oppose synchronous oscillations by using feedback from cortex and the DBS target to adjust the stimulation protocol—show promise as an even more effective method of alleviating motor dysfunction (Rosin et al., 2011). Yet some question whether persistent stimulation is required for therapy or if the “coordinated reset” (CR) of a nucleus or nuclei can ameliorate pathological activity for a longer period of time, as suggested by Adamchic et al. (2014), who observed significant cumulative reductions in beta-band oscillations in PD patients with only 2 brief CR stimulations per day. Mastro et al.’s 2017 results also showed that, with a brief optogenetic elevation of parvalbumin (PV)-expressing GPe neurons over the Lim homeobox 6 (Lhx6)-expressing population (Abdi et al., 2015), acutely parkinsonian mice exhibit motor rescue and reduced pathological burst firing in SNr neurons for hours following stimulation. Thus, the best targets and stimulation protocols for DBS to treat PD are still being discovered and refined, while informing our understanding of circuit mechanisms in BG function and dysfunction.

But DBS is nowhere near an ideal solution for PD treatment: it is invasive, targeting is difficult, and repeated penetration of electrode implants during surgery can be damaging and dangerous.
Furthermore, the incidence of adverse events—from lead site infection to gait disturbances and falls—is about doubled in patients receiving DBS relative to those on their best medical therapy (Weaver et al., 2009; Schuepbach et al., 2013). And while the decorrelation hypothesis may hold true, it is unclear how STN/GPi neuronal excitation (or inhibition, Filali et al., 2004; Toleikis et al., 2012), antidromic stimulation (Kang and Lowery, 2014), or the stimulation of astroglia (Tawfik et al., 2010; Vedam-Mai et al., 2012) or nearby brain structures each contributes to therapy (Breit et al., 2004; Li et al 2007, 2012; Gradinaru et al., 2009; reviewed in Chiken and Nambu, 2016). Basic research in animal models is critical to parse nuances such as these in order to develop and optimize treatments for PD.

Animal models of PD

There is currently no ideal animal model of PD that adequately recapitulates the pathophysiology and symptomology of the idiopathic disease in humans. Familial PD accounts for fewer than 10% of cases, but PD-related mutations such as those in the genes encoding α-synuclein (SNCA) and leucine-rich repeat kinase 2 (LRRK2) proteins have been used to investigate mechanisms that contribute to pathology in both familial and sporadic incidences of PD (Volta et al., 2015). Indeed, neuroprotective and disease-modifying treatments that target putative triggers gleaned from such studies—from calcium dysregulation and mitochondrial dysfunction to neuroinflammation and synucleinopathies in dopamine neurons—show promise (reviewed in AlDakheel et al., 2014). And preclinical biomarkers such as the loss of smell and neuropsychological impairment (reviewed in Berg 2008) are being investigated for their predictive value in diagnosing PD. But while current genetic models are valuable for identifying some of these early pathogenic mechanisms to prevent disease progression, the identified PD-associated mutations alone are seemingly insufficient to trigger DA neurodegeneration in mice to the extent that PD does, failing to produce the magnitude of disability suffered by patients later in the disease. As dopamine neuron degeneration only
becomes clearly suggested when patients develop clinical symptoms—typically after over half of the SNc has succumbed—it is difficult to achieve widespread early intervention to slow the effects of neurodegeneration in preclinical cases. Thus, the majority of patients are still diagnosed after motor symptom onset (Bernheimer et al., 1973; Dauer and Przedborski 2003). Therefore, the use of models that reflect late-stage PD, while not etiologically consistent with the sporadic disease, are valuable for elucidating symptom-associated motor pathologies that we can target for treatment in the majority of diagnosed patients.

The most-studied animal PD models involve neurotoxic lesions to DA neurons and/or their terminal projections—a much quicker and more direct but consequently confounded means of triggering the effects of dopamine depletion in the brain. The mechanism of degeneration common to all the neurotoxic PD models is putatively the formation of reactive oxygen species (ROS) in dopamine neurons, which is also one of the proposed endocellular mechanisms of familial PD (Daur and Przedborski 2003; AlDakheel et al., 2014). But the timescale of degeneration and the accompanying symptoms and biomarkers (or lack thereof) suggest there are aspects of the disease course not adequately captured by these models.

Described by Ungerstedt in 1968, 6-hydroxydopamine (6-OHDA) was the first agent used to target dopaminergic neurons for degeneration. Once injected into the brain (for it cannot cross the blood-brain barrier, BBB), the toxin is preferentially taken up by dopamine and norepinephrine (NE) neurons via their respective transporters due to its similarity in structure to the monoamines (Luthman et al., 1989). Once inside, it produces potentially cytotoxic molecules (e.g. ROS like H$_2$O$_2$) when auto-oxidized or catalyzed by monoamine oxidase (MAO-A; Simola et al., 2007). The resulting oxidative stress, while neurotoxic in itself (Saner and Thoenen 1971; Werner and Cohen, 1993; Blum et al., 2001), may be compounded by mitochondrial dysfunction
through 6-OHDA-mediated inhibition of complex I in the electron transport chain (ETC; Glinka and Youdim, 1995). To model PD, 6-OHDA is injected into striatum, SNc, or MFB accompanied by administration of a norepinephrine transporter (NET) blocker such as desipramine to isolate neurodegenerative effects to dopaminergic neurons. Different targeted denervation protocols are used to produce unilateral or bilateral, acute or chronic, partial or complete dopamine-depleting lesions in rodents, guinea pigs, monkeys, and even cats and dogs (Rothblat and Schneider 1995a, b; Bezard and Przedborski 2011; Tieu 2011).

In idiopathic PD, postmortem patient brains reveal that dopamine loss in the striatum (specifically the dorsolateral putamen of the motor circuit) precedes SNc cell death, reaching 80% dopamine loss in the nigrostriatal terminal field when 60% of the neurons have degenerated and symptoms develop (Bernheimer et al., 1973). Therefore, a lesion protocol that causes DA neuron terminal field degeneration in the striatum before neurodegeneration in the SNc is desirable. While injection of 6-OHDA directly into the SNc causes neurodegeneration within 12 hours (with terminal loss beginning 2-3 days later), MFB and (to a greater extent) striatal injections of the toxin causes a nigrostriatal pathway degeneration pattern more akin to PD (Sauer and Oertel 1994; Przedborski et al., 1995; Sarre et al., 2004). Furthermore, SNc injections of 6-OHDA can produce off-target neurodegeneration of the SNr and ventral tegmental area (VTA), the latter of which shows a relative resistance to degeneration in PD. One suggested mechanism for the selective vulnerability of SNc dopamine neurons is the differential activation of the ATP-sensitive potassium (K\text{ATP}) channel in SNc neurons over VTA, as K\text{ATP} channel knockout was neuroprotective for the SNc in experimental PD models (Liss et al., 2005; Dragicevic et al., 2015; more on K\text{ATP} channels in Section 5.2). Although specific and effective at targeting DA neurons, 6-OHDA lesions in animals do not produce the aggregated protein inclusions (called Lewy bodies, LB) that are usually associated with a PD diagnosis, perhaps indicating that the plasticity and
compensatory changes that accompany gradual deterioration are not engaged in the 6-OHDA model (Meredith et al., 2008). However, LB formation is not specific to PD, and its role in the pathogenesis and symptomology of the disease is still controversial (reviewed in Dauer and Przedborski 2003; Braak et al., 2003). In contrast, the link between Parkinsonism and dopamine system degeneration is well established, and this feature on its own can recapitulate late stage disease phenotypes in animals to give insight into their mechanisms and treatment.

One advantage of intracerebral 6-OHDA injection touted by translational neuroscientists is the ability to produce a unilateral dopamine lesion, creating a hemiparkinsonian model that lends itself to behavioral tests of asymmetry used to score motor function disparities between the dopamine-intact and dopamine-depleted sides (Ungerstedt and Arbuthnott 1970). Specifically, dopamine depletion in one hemisphere causes motor deficits in the contralateral side of the body controlled by the affected brain regions, thus producing “ipsiversive” behaviors that favor the unencumbered (or less-encumbered) side. Though the presence of inter-hemispheric projections precludes the explicit use of the nonlesioned side for “control” comparisons, the hemiparkinsonian symptoms of axial torsion, forelimb use asymmetry, sensory neglect of the contralateral side, and gait disturbances in contralateral limbs added to PD symptoms of reduced spontaneous movement and tremor make the 6-OHDA model a valuable tool for assessing therapies to correct motor dysfunction (Whishaw et al., 1986; Schwarting et al., 1991; Schwarting and Huston 1996; Dowd et al., 2005; reviewed in Bové and Perier 2012). For example, Jungnickel et al. (2011) used the unilateral 6-OHDA model to test the optimal placement of intrastriatal fetal nigral cell grafts to improve spontaneous motor behavior and anxiety in rats. And Rylander et al. (2010) tested the therapeutic benefit of mGluR5 antagonist fenobam using rotorod performance and spontaneous rotation metrics in the unilateral 6-OHDA-based rat model of LID (Lundblad et al., 2004). However, the speed of dopamine depletion in this neurotoxic model and the absence
of LBs leaves something to be desired when searching for a PD model that maps closely to the human disease.

Methylphenyltetrahydropyridine (MPTP) was discovered as a dopaminergic-selective neurotoxin when a handful of heroin users in California unknowingly injected it into their bloodstream (Fahn 1996). The L-DOPA-sensitive bradykinesia that followed suggested whatever he injected induced Parkinsonism. An unintended byproduct of the heroin-like 1-methyl-4-phenyl-4-propionpiperidine (MPPP), MPTP is selectively taken up by the dopamine transporter (DAT) after its catalysis by MAO-B to MPP+, which inhibits complex I of the ETC in mitochondria, putting the cells into metabolic crisis and leading to their demise. This discovery inspired the investigation of mitochondrial dysfunction and oxidant stress as a trigger of neurodegeneration in idiopathic PD, and has led to the development of antioxidant-based interventions in preclinical PD (reviewed in Daur and Przedborski 2003; AlDakheel et al., 2014). MPTP is systemically applied in animal models, and reproduces in monkeys a remarkable set of preclinical and clinical symptoms observed in humans (Bezard and Przedborski 2011). Beyond the obvious similarities in motor symptoms of rigidity and bradykinesia (though not tremor), the MPTP monkey exhibits cognitive impairment (Schneider and Kovelowski 1990; Decamp and Schneider 2004), sleep dysregulation (Barraud et al., 2009), and gastrointestinal dysfunction (Chaumette et al., 2008) in accordance with PD in humans. But, like 6-OHDA models, MPTP administration does not produce LBs (Sian et al., 1999). The herbicide paraquat (N,N′-dimethyl-4-4′-bipiridinium) and the insecticide rotenone have a structure and biological effect similar to MPP+, and results in dopamine neuron loss as well as LB-like inclusions of α-synuclein. However, the effects of paraquat and rotenone on monoaminergic neurons are nonspecific, and paraquat does not easily cross the blood-brain barrier (BBB; Daur and Przedborski 2003; Corasaniti et al., 1992). While their ability to produce Parkinsonism in animals speaks to the environmental risk factors that contribute to PD incidence,
paraquat and rotenone are inadequate as models for the disease. And while MPTP is the current “gold standard” for PD models, it is still relatively acute in its timecourse and is extremely dangerous to researchers, given its ability to cross the BBB (compared with 6-OHDA, which must be directly injected into a dopamine-innervated brain region to exert its neurotoxic effects).

The emergence of new genetic techniques that allow cell- or protein-specific manipulations have made the mouse a highly useful species across research areas, and study of the basal ganglia is no exception. The aforementioned optogenetic experiments selectively targeting D1- vs D2-SPNs (Kravitz et al., 2010) was made possible by the use of transgenic mouse lines. Reporter lines for identifying cell types in live tissue, knockdown techniques, cre-lox expression systems, and genetic disease modeling capabilities make mice a very powerful tool for experimentation that has revolutionized the study of the brain in the last two decades. It is exceptional that we can now do things like express light-sensitive opsins in subsets of neurons identified by activity of a specific promoter and drive their firing in a highly temporally precise manner in an animal that’s awake and walking around and/or performing behavioral tasks (e.g. Kravitz et al., 2010). New imaging techniques are emerging that allow us to “see” the activity in a field of neurons expressing voltage-sensitive fluorescent dyes in addition to optogenetic proteins with non-overlapping excitation spectra (Hochbaum et al., 2014). This use of “optopatch” recording and optogenetic stimulation in behaving mice has the potential to elucidate an incredible amount of information about neuronal activity and its associations with whole-animal outputs. It improves by leaps and bounds our ability to decode the black box that is the brain. Given the powerful toolbox of genetic techniques available in the mouse, and the relative safety, selective potency, well-classified characteristics and behavioral phenotypes achieved by 6-OHDA, this toxin-induced hemiparkinsonian mouse model was used as the primary PD model in this thesis.
Genetic tools are still being utilized to develop new models of PD in an effort to better simulate the progressive degeneration of dopamine neurons experienced by human patients. Genes associated with mitochondrial respiratory chain function have been implicated in familial PD and as risk factors for the idiopathic disease (reviewed in Dexter and Jenner, 2013). A study of substantia nigra tissue from PD patients (Bender et al., 2006) revealed an elevation of mitochondrial (mt)DNA deletions and corresponding reduction in cytochrome oxidase c (Complex IV) activity in the ETC compared with age-matched controls, augmenting the accumulating evidence for respiratory chain dysfunction in SNC neurons found in human patients (Mizuno et al., 1989; Schapira et al., 1990; Hattori et al., 1991). Incidentally, toxins that induce parkinsonism acutely (including 6-OHDA and MPTP) also promote dopamine neuron death by ETC dysfunction, largely by inhibiting Complex I (Schober 2004). Ekstrand et al. (2007) therefore created and characterized a mouse line in which mitochondrial transcription factor A (Tfam) is floxed, and cre-recombinase is selectively expressed under the DAT promoter (Ekstrand and Galter 2009). This produces animals in which mitochondrial transcription factor A (TFAM), a nuclear gene whose product is transported to mitochondria where it is essential for mtDNA transcription, is knocked out in DAT-expressing neurons. The result is the MitoPark mouse, which exhibits stark respiratory deficiencies in midbrain DA neurons, specifically at complex I, starting at 6 weeks of age (Ekstrand et al., 2007). Cytoplasmic inclusions in DA neurons are also observed at 6 weeks, though they appear distinct from Lewy Bodies and comprise mitochondrial membranes but not α-synuclein (Ekstrand et al., 2007). Dopamine neurons and fibers in the caudate/putamen identified by their expression of TH progressively degenerate beginning at 12 weeks in MitoPark mice, with striatal fiber loss progressing ventromedially over time (Ekstrand et al., 2007). Two weeks later, reduced locomotion heralds the progression of PD-like motor impairments that eventually require the animals to be euthanized at 45 weeks of age (Ekstrand and Galter 2009). This new model, while not yet thoroughly characterized, was incorporated into
the present study to corroborate its fundamental findings in a more progressive, genetic model of PD.

1.3 STN activity in PD

Some in vivo studies report an elevation of STN activity in idiopathic and experimental PD (Vila et al., 2000; Tachibana et al., 2011; Bergman et al., 1994; Hassani et al., 1996; Soares et al., 2004; Deffains et al., 2014), as predicted by the rate model of basal ganglia disorders (Albin et al., 1989). However, the autonomously-generated firing observed ex vivo with synaptic transmission pharmacologically inhibited is reduced and irregular in animal models of PD. Zhu et al. (2002a) found that STN neurons from 6-OHDA treated rats exhibited slower and less rhythmic intrinsic activity in slice recordings compared with controls. This result was replicated in MPTP-treated mice by Wilson et al. (2006). The reduction of intrinsic activity may be reflective of a compensatory mechanism that reduces excitability of STN neurons, which would be engaged in the dopamine-depleted brain as the nucleus becomes hyperactive in vivo. Cellular plasticity cascades that suppress a neuron’s excitability and reduce metabolic or oxidative stress are well documented to resist excitotoxicity (Tsitolovsky 2005; Prentice et al., 2015; Rueda et al., 2016). However, the resulting change in firing dynamics in the STN of these PD models—namely a decrease in decorrelating autonomous activity (Wilson 2013) and an increase in synchronized oscillatory activity (Gatev et al., 2006)—suggests an overall negative impact the STN’s ability to integrate synaptic input like it does in the healthy basal ganglia, as it instead becomes enslaved to oscillations imposed by the phase-offset cortical and pallidal inputs. Bezard et al. (2003) review some of the possible compensatory mechanisms engaged in early, presymptomatic PD that don’t involve DA signaling, but rather the plasticity of basal ganglia circuitry that leads to changes in firing rates and correlative activity. The authors emphasize the presymptomatic timing of many
firing changes, which may indicate their compensatory nature. But since neurons can only respond to their environment based on their own input and metabolic and physiological state, activity changes that are adaptive to an individual cell may ultimately be maladaptive in the context of the circuit’s function. This concept of maladaptive plasticity underlies the overarching hypothesis presented herein: namely, that circuit-triggered downregulation of intrinsic activity may be homeostatic, but it ultimately promotes anomalous circuit activity in PD, and its reversal is therapeutic.

In light of the ever-growing literature describing 1) the importance of firing patterns and correlated activity among cortico-basal ganglia-thalamo-cortical elements in health and disease, 2) the impact of intrinsic activity on synaptic integration in the STN, and 3) the overwhelming success of STN DBS to treat PD, the reduction of autonomous firing in Parkinsonian subthalamic neurons suggested by Wilson et al. (2006) and Zhu et al. (2002a) presents an interesting line of questioning. Namely, 1) what are the circuit mechanisms that trigger STN autonomous activity disruption; 2) what are the cellular mechanisms that effect the downregulation in autonomous firing; and 3) does restoration of intrinsic firing reduce oscillatory driving of the STN by its inputs and ameliorate motor dysfunction?

Hallworth et al. (2003) emphasized the importance of studying intrinsic properties of STN activity in health and disease for the development and refinement of interventions based on STN activity modulation. This thesis aims to meaningfully contribute to that effort by answering these questions.
1.4 Specific aims

As life expectancy increases, so does the number of people diagnosed with PD. While neurosurgical and medicinal interventions are being refined and improved, it is important to inform these efforts with basic mechanistic investigations in animal models. The mechanism(s) by which DBS works to alleviate motor symptoms in PD patients is still unclear beyond an association with reduced synchronous oscillatory activity. Observations of disrupted autonomous STN firing in experimental PD may signify the loss of an important decorrelating force in the cortico-basal ganglia-thalamo-cortico circuit. This thesis therefore aims to elucidate the mechanisms and functional consequences of autonomous STN activity disruption in the unilateral 6-OHDA mouse model of PD by experimentally addressing the following hypotheses:

1. Elevated D2-SPN output following loss of dopamine disinhibits STN neurons, leading to elevated STN-NMDAR activation which triggers the downregulation of autonomous firing.

Rationale: Loss of dopaminergic modulation in the striatum leads to hyperactivity of D2-SPN transmission and the release of STN from pallidal inhibition, producing elevated firing of STN neurons in vivo (Filion and Tremblay 1991; Vila et al., 2000; Mallet et al., 2006). Given that STN neurons from PD animals undergo a diminution of autonomous firing when observed ex vivo, the data suggest that hyperactivity observed in vivo is largely driven by synaptic inputs. NMDARs have been shown to mediate abnormal synaptic plasticity in the STN following dopamine depletion (Chu et al., 2015, 2017) and a 1-hr incubation in NMDA suppresses autonomous STN firing in acute brain slices (Atherton et al., 2016). These data suggest that excessive NMDAR activation, facilitated by D2-SPN disinhibition of the STN, may trigger the reduction of autonomous STN activity. This hypothesis is addressed in Chapter 4.
2. Mitochondrial oxidation and K\textsubscript{ATP} channel activation underlie the disruption of autonomous STN activity in PD mice.

Rationale: Excessive Ca\textsuperscript{2+} influx through NMDARs promotes production of mitochondrial ROS by the ETC (Dugan et al., 1995). The ROS H\textsubscript{2}O\textsubscript{2} is a potent chemical messenger that relays information about the cell’s metabolic and oxidative state and effects changes in cellular function (Stowe and Camara, 2009). NMDA and H\textsubscript{2}O\textsubscript{2} have both been shown to activate K\textsubscript{ATP} channels, which can suppress neuronal excitability (Atherton et al., 2016; Shen and Johnson, 2010; Avshalumov et al., 2005). The data supporting this putative mechanism are presented in Chapter 5.

3. Restoration of intrinsic firing with chemogenetic activation of STN neurons reduces the impact of cortico-STN inputs and ameliorates Parkinsonian motor symptoms.

Rationale: Wilson (2013) showed the decorrelating impact of autonomously-generated action potentials on the integration of synaptic input. Furthermore, the propagation of coherent oscillations in PD is reduced by extrinsically stimulating STN neurons with DBS (Benabid et al., 2009; Bronte-Stewart et al., 2009; Eusebio et al., 2012; Whitmer et al., 2012; Agnesi et al., 2013; Trager et al., 2016). These results would predict a reduction in cortico-STN synaptic patterning and an improvement in motor symptoms upon chemogenetic activation of STN neurons to restore intrinsic excitability. These predictions are tested in Chapter 6.
CHAPTER 2: METHODOLOGY

2.1 Animals

Experiments were performed using adult male C57BL/6 (age = 76, 70-92 days; n = 152), Grin1<sup>lox/lox</sup> (B6.129S4-Grin1tm2Stl/J; age = 95, 84-108 days; n = 50), MitoPark (B6.Cg-Tfam<sup>tm1.Neql</sup>/J; age = 130, 130-170 days; n = 9), heterozygous and homozygous adora2a-rM3Ds (B6.Cg-Tg(Adora2a-Chrm3<sup>*</sup>,-mCherry)AD6Blr/J; age = 93, 66-129 days; n = 41), and heterozygous Gabrr3-cre (Tg(Gabrr3-cre)KC112Gsat; age = 68 days; n = 2) mice according to institutional and NIH guidelines for the care and use of animals (Table 2.1.1). Animals were housed in a vivarium singly or with up to 4 other littermates under a 14 hr light, 10 hour dark cycle.
Table 2.1.1 Animal information.

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<th>P value (comparison; test)</th>
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<td></td>
<td>Grin1</td>
<td>50</td>
<td>14 [13, 15]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gabr3-cre</td>
<td>2</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><strong>% striatal TH remaining in ipsilateral hemisphere</strong></td>
<td>Vehicle</td>
<td>80</td>
<td>98 [87, 122]</td>
<td>p &lt; 0.0001 (vehicle vs 6-OHDA; MWU)</td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
<td>98</td>
<td>0.5 [0, 6]</td>
<td></td>
</tr>
</tbody>
</table>
2.2 Surgery

6-OHDA, vehicle, and viral vectors were injected stereotaxically (Neurostar, Tubingen, Germany) under (1–2%) isoflurane anesthesia (Smiths Medical ASD, Inc., Dublin, OH, USA). 6-OHDA (3-4 mg/ml) or vehicle was injected unilaterally into the medial forebrain bundle (MFB; from Bregma: AP -0.7 mm, ML +1.2 mm; from brain surface: DV +4.7 mm; 1.5 µl) to lesion midbrain dopamine neurons or control for injection, respectively. Pargyline (50 mg/kg intraperitoneally, IP) and desipramine (25 mg/kg IP) were used to increase the potency and specificity of 6-OHDA, respectively. In C57BL/6 mice, AAV expressing hSyn-ChR2(H134R)-eYFP (AAV9.hSyn.hChR2(H134R)-eYFP.WPRE.hGH; 1 × 10^{13} GC/ml; University of Pennsylvania Viral Vector Core) was injected at 3 sites in primary motor cortex (AP +0.6 mm, +1.2 mm, and +1.8 mm, ML +1.5 mm; from brain surface: DV +1.0 mm; 300 nl per site), or AAV expressing CMV-MTS-roGFP (AAV9-CMV-MTS-roGFP-SV40; 2.5 × 10^{12} GC/ml; Virovek, Inc., Hayward, CA), hSyn-HA-hM3Dq-IRES-mCitrine (AAV2/5-hSyn-HA-hM3Dq-IRES-mCitrine; 2 × 10^{12} GC/ml), or hSyn-hM3Dq-mCherry (AAV8-hSyn-hM3Dq-mCherry; 2-3 × 10^{12} GC/ml, UNC Vector Core, Chapel Hill, NC; 2-3 x 10^{13} GC/ml, Addgene, Cambridge, MA) was injected in the STN (AP -2.06 mm, ML +1.4 mm, DV +4.45 mm; 300-500 nl) ipsilateral to the MFB injection. In Grin1lox/lox mice AAV expressing eGFP (AAV9-hSyn-eGFP-WPRE-bGH; 2 × 10^{12} GC/ml; University of Pennsylvania Viral Vector Core) or cre-eGFP (AAV9.hSyn.HI.eGFP-Cre.WPRE.SV40; 2 × 10^{12} GC/ml; University of Pennsylvania Viral Vector Core) was injected in the STN (AP -2.06 mm, ML +1.4 mm, DV +4.45 mm; 150-300 nl) ipsilateral to the MFB injection. Gabrr3-cre mice, in which there is cre-recombinase expression throughout the STN but little in adjacent structures, received STN injections of AAV expressing hSyn-DIO-hM3Dq-mCherry (AAV8-hSyn-DIO-hM3Dq-mCherry; 2-3 × 10^{13} GC/ml; UNC Vector Core, Chapel Hill, NC) in a cre-dependent manner. A subset of C57BL/6 mice were injected with AAV8-hSyn-hM3Dq-mCherry dorsal to the STN (AP -
2.06 mm, ML +1.4 mm; DV +3.75 mm) to test the behavioral effects of hM3Dq activation of structures adjacent to but not including the STN. Electrophysiological, imaging and behavioral measurements were made 18, 13-22 days (n = 214) after surgery (Table 2.1.1). Mice received supplemental nutrition and subcutaneous (SC) injections of warm saline (0.5 mL/day) as needed until recovered from surgery.

2.3 Electrophysiology

Ex vivo electrophysiology

Animals were first anesthetized through IP injection of ketamine/xylazine (87/13 mg/kg) and then transcardially perfused with ice-cold sucrose-based artificial cerebro-spinal fluid (sACSF: 230 mM sucrose, 2.5 mM KCl, 1.25 mM NaH2PO4, 0.5 mM CaCl2, 10 mM MgSO4, 10 mM glucose, and 26 mM NaHCO3), equilibrated with 95% O2 and 5% CO2. The brain was then removed and sectioned at 250 µm in the parasagittal plane at 30 µm/s in a chamber containing ice-cold sACSF using a vibratome (VT1200S; Leica Microsystems Inc., Buffalo Grove, IL, USA). Cut slices were transferred to ACSF (126 mM NaCl, 2.5 mM KCl, 1.25 mM NaH2PO4, 2 mM CaCl2, 2 mM MgSO4, 10 mM glucose, 26 mM NaHCO3, 1 mM sodium pyruvate, and 5 µM L-glutathione), equilibrated with 95% O2 and 5% CO2 at 35°C for 30 minutes and then held in artificial CSF at room temperature until recording. For experiments involving MFB injections of vehicle or 6-OHDA, recordings were made from STN neurons in the injected hemisphere.

In the recording chamber, slices were perfused at a rate of 4-5 mL/min with synthetic interstitial fluid (SIF: 126 mM NaCl, 3 mM KCl, 1.25 mM NaH2PO4, 1.6 mM CaCl2, 1.5 mM MgSO4, 10 mM glucose and 26 mM NaHCO3), equilibrated with 95% O2 and 5% CO2 at 35°C. A microscope (Axioskop FS2; Carl Zeiss, Oberkochen, Germany) equipped with a LUMPlanFl/IR 60 X 0.9 NA
objective (Olympus, Tokyo, Japan) or a BX51WI microscope (Olympus) equipped with a UIS1 LUMPFL 60 × 0.9 NA objective (Olympus)) employing infrared Dodt Gradient Contrast illumination (Luigs & Neumann, Ratingen, Germany) was used to visualize cell bodies for patch clamp recording. 590 nm LED illumination (Cairn Instruments, Faversham, Kent, UK) was used to identify neurons expressing hM3Dq-mCherry and 890 nm 2-photon illumination (Coherent Inc., Santa Clara, USA; as for MTS-roGFP imaging, detailed below) was used to identify neurons expressing eGFP or cre-eGFP.

Recordings were obtained using computer-controlled manipulators (Luigs & Neumann) and a Multiclamp 700B amplifier, and a Digidata 1440A digitizer controlled by PClamp10 (Molecular Devices, Sunnyvale, CA, USA). Signals were low-pass filtered online at 10 kHz and sampled at 50 kHz. Cell and electrode capacitances and series resistance were compensated electronically. Junction potential correction for whole-cell current clamp recordings was done offline. Recordings utilized borosilicate glass pipettes (Warner Instruments, Hamden, CT, USA) pulled using a P-97 Flaming/Brown Micropipette Puller (Sutter Instruments, Novato, CA, USA). Loose-seal cell-attached recordings were made with 3-5 MΩ impedance pipettes containing HEPES-buffered SIF solution (HBS: 140 mM NaCl, 23 mM glucose, 15 mM HEPES, 3 mM KCl, 1.5 mM MgCl₂, 1.6 mM CaCl₂; pH 7.2 with NaOH; 300–310 mOsm/L). Cell-attached recordings were excluded if the membrane became disrupted. Whole-cell voltage clamp recordings were made using 4-5 MΩ impedance pipettes filled with a K-gluconate-based internal solution (140 mM K-gluconate, 3.8 mM NaCl, 1 mM MgCl₂, 10 mM HEPES, 0.1 mM Na₄-EGTA, 0.4 mM Na₃GTP, and 2 mM Mg₁.₅ATP). Voltage clamp recordings were excluded if series resistance changed by more than 20% during the course of recording. Whole-cell current clamp recordings were made using 12-15 MΩ impedance pipettes filled with K-MeSO₄-based internal solution (130 mM KCH₃SO₄,
3.8 mM NaCl, 1 mM MgCl$_2$, 10 mM HEPES, 5 mM phosphocreatine, 0.1 mM Na$_4$-EGTA, 0.4 mM Na$_3$GTP, and 2 mM Mg$_{1.5}$ATP).

Optogenetic stimulation was delivered via the objective lens using a 470 nm light emitting diode (OptoLED; Cairn Research, Faversham, Kent, UK). Cortico-STN transmission was evoked by 1 ms duration optogenetic stimulation of cortico- STN axon terminals. The specific pattern of stimulation is detailed in the paper. EPSCs and IPSCs were also evoked by electrical stimulation of the internal capsule bordering the rostro-ventral STN using a constant current isolator (A365R, World Precision Instruments, Sarasota, FL). The poles of stimulation were selected from a custom-built matrix of 10 stimulation electrodes (MX52CBWMB2, Frederick Haer, Bowdoin, ME).

Autonomous activity was typically recorded in the presence of 20 µM DNQX, 50 µM D-APV, 10 µM SR-95531, and 2 µM CGP55845 (Abcam, Cambridge, MA, USA) to antagonize AMPARs, NMDARs, GABA$_A$Rs and GABA$_B$Rs, respectively. DNQX and D-APV were excluded when the response of STN neurons to optogenetic cortico-STN stimulation or electrically evoked EPSCs were studied. SR-95531 and CGP55845 were excluded when electrically evoked IPSCs were studied. The effect of catalase (polyethylene glycol-catalase; 250 U/mL; Sigma-Aldrich), glibenclamide (100 nM; Sigma-Aldrich), apamin (10 nM; Sigma-Aldrich), or CNO (10-100 µM; Sigma-Aldrich) on firing was also studied.

In vivo electrophysiology
Adora2a-rM3Ds mice were first injected with urethane (1.24 g/kg, IP) and then 60 min later with supplements (up to 0.49 g/kg additional, IP) every 20 min until the toe-pin counts withdrawal reflex was abolished. Mice were then placed in a stereotaxic frame (David Kopf Instruments, Tujunga,
for the duration of the recording session with further urethane supplementation, as required. Craniotomies were drilled over the motor cortex (coordinates: AP: +1.4, ML: +1.5mm) and GPe (AP: -0.1, ML: +1.7 mm) and irrigated with HEPES-buffered saline during electrode penetration. EEG signals and single unit GPe activity were acquired from a peridural skull screw implanted over M1 and a silicon tetrode array in the GPe, respectively (NeuroNexus Technologies, Ann Arbor, MI), with a reference wire implanted in the ipsilateral temporal musculature. Recordings were made using a 64-channel Digital Lynx (Neuralynx, Bozeman, MT) data acquisition system at a sampling rate of 40 kHz. The EEG was filtered between 0-400 Hz and single unit activity was bandpass filtered during acquisition between 200-9,000 Hz. Following establishment of cortical slow-wave activity and isolation of single unit GPe activity, baseline activity was recorded for 15-20 min. 1 mg/kg CNO was then administered (SC) to activate rM3Ds-expressing D2-SPNs and recording continued for at least 45 min post-injection. Finally, mice were administered ketamine/xylazine (87/13 mg/kg, IP), perfuse fixed in 4% paraformaldehyde in 0.1M PB, pH 7.4 and post-fixed overnight, as described in Section 2.6. Seventy mm-thick coronal sections were taken using a vibratome (VT 1000S; Leica Biosystems Inc.) to visualize the sites of recording. Silicon probes were immersed prior to use in lipophilic florescent dye (DiI, 20 mg/ml in 50% acetone/methanol) to facilitate reconstruction of recording sites. A combination of template matching, principal component analysis, and manual clustering were used to isolate single unit activity (Plexon Offline Sorter; Plexon, Dallas, Texas).

2.4 2-photon imaging of MTS-roGFP

Brain slices were prepared from mice expressing the mitochondrially-targeted redox-sensitive probe MTS-roGFP in STN neurons, as for electrophysiology. MTS-roGFP-expressing neurons were imaged at 890 nm with 76 MHz pulse repetition and ~250 fs pulse duration at the sample
plane. Two-photon excitation was provided by a G8 OSL pumped Mira 900 F laser (Coherent, Santa Clara, CA, USA). Sample power was regulated by a Pockels cell electro-optic modulator (model M350-50-02-BK, Con Optics, Danbury, CT, USA). Images were acquired using an Ultima 2P system running PrairieView 5 (Bruker Nano Fluorescence Microscopy, Middleton, WI, USA) and a BX51WI microscope (Olympus, Tokyo, Japan) with a 60 × 0.9 NA objective (UIS1 LUMPFL; Olympus). MTS-roGFP fluorescence and Dodt contrast images were acquired from each field of STN neurons in the presence of 20 µM DNQX, 50 µM APV, 10 µM SR-95531, and 2 µM CGP55845 under baseline conditions, following the addition of 2 mM dithiothreitol (DTT) to fully reduce the tissue, and following the addition of 200 µM aldrithiol to fully oxidize the tissue (Hanson et al., 2004). Average fluorescence intensities of mitochondria in STN neurons under control, reduced, and oxidized conditions were quantified using Image J (NIH, Bethesda, MD, USA). Baseline mitochondrial oxidation was then expressed relative to that under conditions of minimum and maximum oxidation.

2.5 Behavioral testing

To assess forelimb use asymmetry, animals were placed in a 600 ml cylindrical glass beaker (9.5 cm diameter; 12 cm height) and imaged using an HD digital camcorder recording at 60 fps (VIXIA HF R40 Full HD Camcorder; Canon, Melville, NY, USA). Forepaw placements on the vertical walls of the beaker were counted manually during video review and forelimb use preference calculated (Schallert et al., 2000). Placement of the left and right forepaw were considered simultaneous (“both”) if they occurred within 3 frames of each other (i.e. < 33 ms apart). Mice expressing hM3Dq in the STN or adjacent structures (or no virus to control for off-target effects of CNO) were tested ~ 20-60 minutes following SC injection of vehicle or 1 mg/kg clozapine-n-oxide (CNO) or in the absence of injection.
Spontaneous locomotor activity was tested in *Grin1* mice using a 40 cm x 40 cm open field chamber with dark, infrared-penetrable walls, a suspended camera fitted with an infrared filter, and ANY-maze software (Stoelting Co., Wood Dale, IL, USA). Recordings were made under infrared illumination for 5 minutes. The first 2 minutes of activity were discarded to exclude novelty-induced exploration. Mice that underwent both behavioral assessments were observed in the open field prior to the forelimb use asymmetry test to eliminate the impact of cylinder exposure on spontaneous movement in the open field. Animals were tested within 2 hours of the commencement of the animals’ dark cycle.

### 2.6 Histology

In order to study the expression of ChR2(H134)-eYFP, cre-eGFP, eGFP, rM3D(Gs)-mCherry, MTS-roGFP, hM3Dq-mCherry, c-fos, NeuN, PV, and tyrosine hydroxylase brain tissue was first fixed in 4% paraformaldehyde in 0.1M phosphate buffer, pH7.4. Tissue prepared for electrophysiology or MTS-roGFP imaging was immersion-fixed. Mice used for behavioral analyses were perfused-fixed under deep anesthesia (IP: 87 mg/kg ketamine, 13 mg/kg xylazine). Tissue was held in fixative at 4°C for at least 12 hours before rinsing in phosphate buffered saline (PBS; 0.05M; pH 7.4). Tissue exceeding 250 µm in thickness was re-sectioned at 70 µm using a vibratome (Leica VT1000S; Leica Microsystems Inc.). Immunohistochemical detection of c-fos, NeuN, PV, and TH was carried out in PBS containing 0.5% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) and 2% normal donkey serum (Jackson ImmunoResearch, West Grove, PA, USA). Tissue was incubated in primary antibodies for 48-72 hours at 4°C or overnight at room temperature (rabbit anti-c-fos: 1:500 dilution; cat# 2250S; Cell Signaling Technology, Danvers, MA; mouse anti-NeuN: 1:200 dilution; cat# MAB377; EMD Millipore, Darmstadt, Germany;
guinea pig anti-PV: 1:1,000; cat# 195 004; Synaptic Systems, Gottingen, Germany; mouse anti-TH: 1:500 dilution; cat# MAB318; EMD Millipore), washed in PBS, and then incubated in their respective secondary antibodies (donkey anti-rabbit Alexa Fluor 488; dilution 1:250; cat # 711-545-152; Jackson ImmunoResearch; donkey anti-mouse Alexa Fluor 488; dilution 1:250; cat # 715-545-150; Jackson ImmunoResearch; donkey anti-mouse Alexa Fluor 594; dilution 1:250; cat # 715-585-150; Jackson ImmunoResearch; donkey anti-guinea pig Alexa Fluor 647; dilution 1:250; cat # 706-605-148; Jackson ImmunoResearch) for 90-120 minutes at room temperature before a final wash with PBS. All tissue was mounted on glass slides using ProLong Diamond Antifade Reagent (ThermoFisher Scientific, Waltham, MA, USA) and coverslipped. Sections were imaged using an Axioskop 2 microscope (Carl Zeiss) equipped with a Neurolucida system (MBF Bioscience, Williston, VT, USA) and/or a confocal laser scanning microscope (A1R; Nikon, Melville, USA).

The densities of c-fos-positive neurons were assessed using NIH ImageJ and quantified using the optical dissector method. Confocal images were collected using a 60x lens on a Nikon A1R confocal microscope (NA=1.4, 0.205 micron/pixel; Nikon, Melville, USA). Sample sites were chosen using a grid (frame size, 50 X 50 micron) that was superimposed randomly on each image stack. Stereological counting commenced and was terminated at an optical section 5 µm and 21 µm below the slice surface, respectively. Look-up and reference planes were separated by 2 µm.

Dopaminergic innervation was assessed from tyrosine hydroxylase (TH) immunoreactivity as described previously (Fan et al. 2012; Figure 3.1.1). Briefly, the average brightness of striatal TH immunoreactivity following subtraction of background fluorescence in the overlying cortex was compared between hemispheres and the % of TH immunoreactivity remaining on the dopamine-depleted side was calculated. In the 62 vehicle-injected and 98 6-OHDA-injected mice used in this
study ipsilateral striatal TH immunoreactivities were 98, 87-122% and 0.5, 0-6% of the contralateral hemisphere, respectively (Table 2.2.1; p < 0.05).

2.7 Data analysis and statistics

Box-and-whisker plots displaying medians with inter-quartile and 10-90% ranges or tilted line segment plots displaying paired data were produced in Prism 7 to reflect the data’s distribution and central tendency (GraphPad Software, Inc., La Jolla, CA). No assumption of normality was made, and thus non-parametric (two-sided) statistics were used for all analyses (Mann-Whitney U (MWU) test for unpaired data, Wilcoxon signed rank (WSR) test for paired comparisons, and Fisher’s exact test for contingency analyses; exact p values were calculated using the fisher.test and the Wilcox.exact function (exactRankTests package, https://CRAN.R-project.org/package=exactRankTests) in R (R foundation for Statistical Computing, Vienna, Austria)). Where datasets were used for multiple comparisons the p-value was adjusted to maintain the family-wise error rate at 0.05 using the Holm-Bonferroni method (Holm, 1979; notated pm). The investigator was unaware of viral expression status when scoring behavior. No specific randomization methods were used to assign animals to treatment groups. For the primary findings reported in the manuscript, sample sizes for MWU and WSR tests were estimated to achieve a minimum of 80% power using formulae described by Noether (1987). The effect sizes used in these power calculations were estimated using data randomly drawn from uniform distributions (runif() function in R stats package). For MWU tests, with a 50 percentile change in median between groups X and Y (the inter-quartile ranges of the groups don’t overlap) P(Y > X) ≈ 0.88 giving an estimation that at least 10 observations per group would be needed to achieve 80% power; for a 25 percentile change (the median of Y falls outside the interquartile range of X) P(Y > X) ≈ 0.72 and the estimated requirement is at least 27 observations per group. For WSR
tests, if all pairs of observations show the same direction of change, \( P(X + X' > 0) = 1 \) giving an estimation that at least 10 observations would be needed to achieve 80% power (note though that it is possible to show empirically that 6 observations gives 100% power in this case); if 90% of observations show the same direction of change, \( P(X + X' > 0) \approx 0.98 \) and the estimated requirement is at least 12 pairs of observations.
CHAPTER 3: AUTONOMOUS STN FIRING IN MOUSE MODELS OF PD

3.1 Disruption of autonomous STN activity

3.1 Introduction

The use of mice in basic science research affords access to an ever-growing genetic tool kit that enables cell-type specific targeting, manipulation, and in situ measurements. Given the risks of working with MPTP and the depth of literature characterizing the 6-OHDA mouse model, the latter was used for the majority of contained studies; the foundational observations were also replicated in symptomatic (20-week-old) MitoPark mice, of which we had access to a limited number. It should be noted that, while the GPi is classified as the entopeduncular nucleus (EP) in rodents and cats, this structure will herein be called the GPi for ease of understanding and drawing comparisons to the human disease.

Reports of a reduction in rate and regularity of autonomous STN activity in experimental PD (Zhu et al., 2002a; Wilson et al., 2006) revealed an interesting caveat to in vivo recordings showing an elevation of firing and increase in bursts (Vila et al., 2000; Tachibana et al., 2011; Bergman et al., 1994; Hassani et al., 1996; Soares et al., 2004; Deffains et al., 2014). Given the decorrelating influence of intrinsic firing on synaptic integration (discussed in Section 1.1; Wilson 2013), it would follow that its disruption or loss may promote entrainment of the STN to synchronous circuit oscillations in PD (discussed in Section 1.2). Our interests lie in the activity of motor circuit STN neurons, which were targeted either by positive identification through optogenetic
stimulation of motor cortico-STN terminals, or by restricting recordings to the lateral half of the
nucleus (Wichmann et al., 1994). To begin, we sought to reproduce in the unilateral 6-OHDA and
MitoPark mouse models the results of Zhu et al. (2002a) and Wilson et al. (2006) which
demonstrated a disruption of intrinsic STN activity in the 6-OHDA rat and MPTP mouse,
respectively.

3.1 Experimental Approach

Animal preparation

In adult C57BL/6 mice, stereotactically-guided injections (Section 2.2) were made, in the same
hemisphere, of: 1) 6-OHDA or vehicle solution into the MFB; and 2) ChR2-eYFP into M1 (motor
cortex). 2-3 weeks following surgery, 250-µm sagittal brain slices were prepared in the dark for
electrophysiological recording (Section 2.3).

Autonomous STN activity recordings

Neurons from C57BL/6 mice that received MFB injections of vehicle or 6-OHDA were
optogenetically identified as receiving input from M1 with a 1 s, 20 Hz stimulation with a 470 nm
LED to excite ChR2-positive axon terminals in the area of the recorded cell. Cells in which
stimulation elicited synaptically-driven APs (i.e. if the number of APs per 10 ms bin during the
stimulation period ever exceeded the mean + 3SD APs per 10 ms bin during the pre-stimulus
period) were deemed M1-connected with 99.7% confidence and were included in this survey of
STN neurons of the motor circuit.

STN neurons from MFB 6-OHDA- or vehicle-injected mice with M1 ChR2 expression were
recorded in the cell-attached configuration in the presence of SR-95531 and CGP55845 to block
GABA_A and GABA_B-mediated synaptic transmission, respectively. For this experiment, blockers
of excitatory synaptic transmission were omitted to allow for optogenetic identification. However, while intrinsically active and relatively local GPe inputs may still be inhibiting STN neurons in the slice, glutamatergic projection neurons that synapse in the STN have been severed from their circuit and, unstimulated, do not transmit glutamate appreciably in the slice. This justification is supported by results in Wilson et al. (2006). Lateral STN neurons from MitoPark mice and their littermate controls were recorded in the presence of inhibitors of GABAergic transmission as well as DNQX and APV to block AMPA and NMDAR-mediated glutamatergic transmission, respectively.

3.1 Results

Autonomous STN activity is downregulated following loss of dopamine

When compared with control tissue, STN neurons from 6-OHDA-injected animals exhibited a reduction in the average frequency (1/inter-spike interval) and regularity (coefficient of variation (CV) = standard deviation/mean inter-spike interval) of autonomous activity (Figure 3.1.1a-d; Table 3.1.1). Additionally, the proportion of autonomously active STN neurons was reduced by ~25% in 6-OHDA-injected animals compared to controls (Figure 3.1.1h; Table 3.1.1). The frequency, regularity, and incidence of autonomous firing was also reduced in 20 week-old MitoPark mice (in which striatal dopamine loss has exceeded 95%; Ekstrand et al., 2007) when compared with littermate controls (Figure 3.1.1e-h). These data confirm in both the unilateral 6-OHDA and MitoPark mouse models the intrinsic STN firing disruption observed in other animal models of PD (Zhu et al., 2002a; Wilson et al., 2006).
Figure 3.1.1. Autonomous STN activity is disrupted in M1-connected neurons from 6-OHDA-injected mice and in MitoPark mice. (a) Tyrosine hydroxylase (TH) immunoreactivity (red) and expression of ChR2-eYFP (green) ipsilateral and contralateral to injections of 6-OHDA in the MFB and an AAV vector expressing ChR2-eYFP in the primary motor cortex (M1) (b) ChR2-eYFP (green)-expressing motor cortico-STN axon terminals in the vicinity of NeuN-immunoreactive (red) neurons (asterisks) in the ipsilateral STN following the injections described above. (c, d) The frequency and regularity of autonomous activity in STN neurons that receive motor cortical input was reduced in slices from dopamine-depleted mice compared to dopamine-intact control mice (c, examples traces; c insets, response to optogenetic stimulation (blue) of motor cortical input; d, population data). Neurons were considered M1-connected if optogenetic stimulation elicited greater than the average + 3SD spikes/bin recorded during the pre-stimulus period. (e) TH immunoreactivity (red) in 20 week-old control and MitoPark mice (green, NeuN-immunoreactive neurons). (f, g) The frequency and regularity of autonomous activity in STN neurons in slices from MitoPark mice was reduced relative to age-matched control mice (f, examples traces; g population data). (h) The incidence of autonomous STN activity was significantly reduced in 6-OHDA-injected and MitoPark mice relative to dopamine-intact control mice. * p < 0.05. n.s., not significant.
Table 3.3.1. Autonomous STN activity is disrupted in M1-connected neurons from 6-OHDA-injected mice and in MitoPark mice (relates to Figure 3.3.1)

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<th>value</th>
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<td>4/66</td>
<td>0.37 [0.23, 0.99]</td>
<td>p &lt; 0.0045 (WT vs mitoPark; MWU)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Active</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>5/105</td>
<td>81%</td>
<td>p &lt; 0.016 (WT vs mitoPark; Fisher's)</td>
<td></td>
<td></td>
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<tr>
<td>MitoPark</td>
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<td>65%</td>
<td>p &lt; 0.016 (WT vs mitoPark; Fisher's)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2 Conclusions

The persistence of autonomous STN activity disruption across at least four PD models suggests its potential salience in the pathophysiology and symptomology of the disease. But STN activity is increased in the dopamine-depleted brain in vivo (e.g. Vila et al., 2000; Tachibana et al., 2011; Bergman et al., 1994; Hassani et al., 1996; Soares et al., 2004; Deffains et al., 2014), implicating elevated synaptic excitation as the driving force of the cell’s firing in the intact brain. The reduction of STN excitability (per its downregulation of autonomous activity) may therefore be a homeostatic response to resist excitotoxicity in the face of elevated glutamatergic input following dopamine loss. Maintaining a membrane potential is energetically costly—especially in intrinsically active and/or prolifically stimulated neurons. Adequate levels of ATP are required to sustain the electrochemical gradient across the membrane by driving the Na⁺/K⁺ pump, for one; failure of this mechanism is disruptive and ultimately deadly to the cell. Interestingly, Vaarmann et al. (2013) observed that DA receptor activation in neurons is neuroprotective against glutamate excitotoxicity: in primary neuronal cultures from cortex, hippocampus, and the midbrain, low (0.5 – 1 µM) concentrations of dopamine tempered the initial rise in cytosolic Ca²⁺ induced by glutamate application (5 µM) and abolished the delayed calcium deregulation, reducing cell death in a glutamate toxicity paradigm (more on this in Section 5.1). While STN neurons do receive DA input that is lost in PD, they do not appear to degenerate in the 6-OHDA rodent model (Carvalho and Nikkhah, 2001). Instead it appears that non-lethal homeostatic triggers engage mechanisms in the dopamine-depleted brain that alter cellular and synaptic plasticity to promote cell survival. This thesis proposes a mechanism of cellular plasticity in the STN to account for the disruption of autonomous firing that may be homeostatic in nature, but ultimately maladaptive in the context of the Parkinsonian circuit.
CHAPTER 4: CIRCUIT TRIGGERS OF AUTONOMOUS STN ACTIVITY DISRUPTION

4.1: D2-SPN hyperactivity

4.1 Introduction

Dopamine loss in the STN and striatum

When considering potential triggers of autonomous STN activity disruption in experimental PD, the most obvious candidate is the loss of dopamine itself. As described in Section 1.1, the STN does receive dopaminergic input from the SNc, and these afferents do degenerate in PD (Hassani et al., 1997; François et al 2000). Indeed, DA has been shown to acutely increase intrinsic activity in STN neurons ex vivo (Ramanathan et al., 2008; Loucif et al., 2008; Zhu et al., 2002a; Cragg et al., 2004). But when Ni et al. (2001) injected 6-OHDA into the STN to selectively degenerate SNc terminals there, 1) rats did not exhibit apomorphine-induced rotational behavior (Waddington and Crow, 1978), 2) in vivo STN recordings of anesthetized rats exhibited only slightly elevated bursting activity (an 11% increase over controls compared with 64% in PD rats), and 3) overall firing rates of STN neurons decreased, while STN firing in vivo is consistently increased in PD and its models (Vila et al., 2000; Tachibana et al., 2011; Bergman et al., 1994; Hassani et al., 1996; Soares et al., 2004; Deffains et al., 2014). Together these findings suggest that dopamine loss in the STN itself is insufficient to reproduce PD-like firing and behavior, and upstream striatal DA depletion is likely responsible for STN activity perturbations in parkinsonism. As discussed in Section 1.2, dopamine loss results in a relative hyperactivity of the indirect pathway (Filion and Tremblay 1991; Mallet et al., 2006). We therefore sought to test whether chronic activation of D2-
SPN output (to simulate their hyperactivity in the dopamine-depleted state) alone was sufficient to trigger plasticity in the STN in a dopamine-intact brain. As a proxy for dopamine loss isolated to the indirect pathway, we selectively activated adora2a-expressing neurons in vivo to simulate PD-like increase in D2-SPN excitability using a revolutionary tool for extrinsic neuromodulation: chemogenetics (Coward et al., 1998; Lee et al., 2014; Smith et al., 2016).

Chemogenetics

In 2007 Bryan Roth’s group developed a new kind of receptor activated solely by synthetic ligands (RASSL) and named it a designer receptor exclusively activated by designer drugs (DREADD; Armbruster et al., 2007; Nawarante et al., 2008; Conklin et al., 2008; Dong et al., 2010). They used yeast and directed molecular evolution of different human muscarinic receptor (hM) subtypes such that the new receptors would no longer be responsive to their native agonists, but instead to a synthetic ligand: clozapine-n-oxide (CNO; Dong et al., 2010; reviewed by Urban and Roth 2015). DREADDs, when expressed virally or in transgenic animal lines, allow the targeted engagement of G protein-coupled receptor (GPCR) signaling with a non-endogenous activator that putatively has no effect on other receptors in the body (though other more specific ligands are being developed amid reports of off-target effects of CNO metabolites in non-murine species; MacLaren et al., 2016; Chen et al., 2015; see Section 7.4 for additional discussion of CNO in vivo). G<sub>q</sub>-, G<sub>i/o</sub>-, and G<sub>s</sub>-linked DREADDS were evolved from hM4, hM3, and hM3/β-adrenergic hybrid receptors, respectively (Urban and Roth 2015). Each harnesses the corresponding intracellular signaling pathways endemic to the target cells, allowing a variety of modulatory power with a receptor prevalence- and CNO dose-dependence: G<sub>q</sub> DREADDs (hM3Dq) elevate excitability in most cells, often producing burst-like firing (e.g. Krashes et al., 2011); G<sub>i/o</sub> DREADDs (hM4Di) inhibit activity, typically through inwardly-rectifying K<sup>+</sup> (GIRK) channels (Armbruster et al., 2007; Wei et al., 2017); and G<sub>s</sub> DREADDs (rM3Ds), whose activation elevates
cAMP production and DARPP-32 phosphorylation and depolarizes cells (Becnel et al., 2013; Farrell et al., 2013; Aitta-aho et al., 2016; Parfitt et al., 2017). Of these three, mild constitutive activity has only been reported for Gs signaling (Guettier et al., 2009). Even so, robust metabolic and behavioral effects are still observed upon its activation with CNO (Guettier et al., 2009; Farrell et al., 2013).

In 2013, the Roth lab published their development and characterization of a transgenic mouse that expresses the rM3Ds receptor under the adora2a promoter (for the adenosine A2A receptor), which is predominantly active in the SPNs of the indirect pathway (Chen et al., 2001; Farrell et al., 2013). Consistent with optogenetic experiments to selectively stimulate D2-SPNs (e.g. Kravitz et al., 2010), CNO administration to adora2a-rM3Ds transgenic mice produced a pronounced reduction in spontaneous locomotion, thereby appearing to recapitulate the hyperactivity of the indirect pathway and associated akinesia of PD (Farrell et al., 2013; Chu et al., 2017). Complementary experiments by Lemos et al. (2016) also demonstrated that dopamine-intact rats lacking D2Rs in D2-SPNs reproduce PD-like motor phenotypes. We therefore used the adora2a-rM3Ds mouse to first characterize the impact of chemogenetic activation on electrophysiological measures of AP firing and synaptic transmission, and if consistent with our assumptions, determine whether selective chemogenetic stimulation of D2-SPNs in dopamine-intact animals for 3 days is sufficient to trigger PD-like autonomous STN activity disruption. Testing this hypothesis will also address whether the loss of nigro-subthalamic DA modulation is necessary for pathogenesis of this STN phenotype.
4.1 Experimental Approach

Animal preparation

To measure the acute physiological effects of rM3Ds activation in D2-SPNs, brain slices were prepared from naïve adult male 2-copy adora2a-rM3Ds mice. The acute and persistent behavioral impacts of CNO (1 mg/kg) were also measured in 2-copy adora2a-rM3Ds animals by recording open field activity before and 30 minutes after injection of either vehicle or CNO on 3 consecutive days. (N.B.: This frequency of injection in 2-copy mice led to a high instance of mortality; thus, 1-copy animals were used in subsequent experiments employing chronic CNO treatment.) To mimic the chronic elevation in D2-SPN inhibition following dopamine denervation and determine its impact on autonomous STN activity, adult male 1-copy adora2a-rM3Ds mice were injected with CNO (1 mg/kg) subcutaneously once every 12 hours for 3 days, totaling 6 injections. Adora2a-rM3Ds mice recorded following the same regimen of vehicle injections served as controls.

Electrophysiological recordings

To measure the impact of chemogenetic activation on the excitability of D2-SPNs, APs were measured during current injections of 0, 200, and 400 pA during control conditions and with 10 µM CNO in slices from naïve adora2a-rM3Ds mice. Ex vivo voltage clamp recordings of miniature (m) IPSCs in GPe neurons were carried out in CNO-perfused and control slices from adora2a-rM3Ds mice in the presence of 1 µM tetrototoxin to determine the effect of chemogenetic activation on synaptic transmission at the striatopallidal synapse. GPe recordings were made in urethane-anesthetized adora2a-rM3Ds mice during slow-wave activity under control conditions and after the administration of CNO (1 mg/kg). To test whether D2-SPN activation alone can phenocopy PD-like activity, 3 hours following the final injection of CNO or vehicle in the adora-rM3Ds mice of the chronic activation paradigm, electrophysiological slices were prepared and cell-attached recordings of autonomous firing of STN neurons were carried out.
4.1 Results

Confirmation of acute adora2A-rM3Ds action

To confirm the modulatory effects of adora2a-rM3Ds activation on basal ganglia circuit activity, several measures were assessed under control conditions and following chemogenetic activation with CNO (1 mg/kg in vivo; 10 µM ex vivo): 1) the number of action potentials fired by striatal rM3Ds-mCherry-expressing neurons in response to depolarizing voltage steps during ex vivo whole-cell current clamp recording; 2) the frequency and amplitude of miniature (m) IPSCs in GPe neurons ex vivo; 3) the frequency of GPe firing in urethane-anesthetized mice; and 4) spontaneous locomotion in an open field. While bath application of CNO did not alter the number of APs evoked by current injection in D2-SPNs (Figure 4.1.1d, e), chemogenetic activation ex vivo did increase the frequency, but not the amplitude of mIPSCs in GPe neurons (Figure 4.1.1f-h; Table 4.1.1). These data are consistent with an elevation of GABAergic striatal transmission to the GPe in response to chemogenetic activation of D2-SPNs. Indeed, in vivo recordings of GPe activity during slow wave cortical oscillations confirmed that SC CNO injection acutely decreased the firing frequency of GPe units in adora2a-rM3Ds mice (Figure 4.1.1i, j; Table 4.1.1). Together these experiments provide proof of concept for chemogenetically elevating D2-SPN-GPe inhibition (and presumably disinhibiting the STN) to mimic Parkinsonian circuit activity changes. Furthermore, consistent with the results of Farrell et al., 2013, chronic in vivo activation of adora2a-rM3Ds for 3 days resulted in a drastic, PD-like reduction of spontaneous locomotion in the open field compared with vehicle-injected littermates (Figure 4.1.1k-m; Table 4.1.1). The akinetic effect of CNO was apparent both acutely (Figure 4.1.1k, l) and persistently when compared with vehicle-injected controls (Figure 4.1.1m).
Figure 4.1.1 Adora2a-rM3Ds activation increases striatopallidal transmission and reduces locomotion. (a–c) Expression of rM3Ds-mCherry in D2-SPNs (a, red arrows) and their axon terminal fields in the GPe (b) in the homozygous adora2A-rM3Ds mouse. Expression was absent in putative D1-SPNs (a, white arrows) and their axon terminal fields in the SNr (c). (b, c) Expression was also absent in neurons of the GPe (b, white arrows) and SNr (c, white arrows). Immunohistochemistry for NeuN (white) was used as a neuronal marker in (a)–(c). (d, e) The presence of CNO (10 µM) in the bath to activate adora2a-rM3Ds during whole-cell patch clamp recording did not change the excitability of mCherry-expressing D2-SPN neurons in response to depolarizing current injections, per the number of APs elicited by each (d, example traces; e, population data). (f, g) The frequency (f and g) but not the amplitude (f and h) of mIPSCs in GPe neurons was greater in brain slices treated with CNO (10 µM) ex vivo versus untreated control slices (f, representative examples; g and h, population data). (i, j) Simultaneous recordings of the electroencephalogram (EEG) band pass filtered at 0.5–1.5 Hz and 10–100 Hz, and GPe unit activity in a urethane-anesthetized adora2A-rM3Ds mouse prior to (control), and 30–45 min following the SC administration of CNO (1 mg/kg). The rate of GPe unit activity during periods of robust cortical slow-wave activity decreased following the injection of CNO both in each example neuron (i) and across the population sample (j). (k-m) Chemogenetic activation of rM3Ds in D2-SPNs through SC injection of CNO (1 mg/kg) led to inhibition of open field motor activity relative to vehicle injection. (k) Representative examples of open field activity before and after first injection. (l and m) Population data confirming that CNO injection reduced movement traveled in the open field (l, left and right boxplots for vehicle and CNO represent movement prior to and following first injection, respectively; m, movement following injection of vehicle or CNO over 3 consecutive days). Figure panels reproduced with permission from Chu et al., 2017.
Table 4.1.1 Adora2a-rM3Ds activation increases striatopallidal transmission and reduces locomotion (relates to Figure 4.1.1).

<table>
<thead>
<tr>
<th>Value</th>
<th>Condition</th>
<th>Treatment group</th>
<th>n (mice/cells)</th>
<th>Median [IQ range]</th>
<th>p value (comparison; test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 pA</td>
<td>A2A/saline</td>
<td>2/8</td>
<td>0 [0, 0]</td>
<td></td>
<td>p &lt; 0.9999 (APs in saline vs CNO at 0 pA; MWU)</td>
</tr>
<tr>
<td></td>
<td>A2A/CNO</td>
<td>2/9</td>
<td>0 [0, 0]</td>
<td></td>
<td>p &lt; 0.9999 (APs in saline vs CNO at 100 pA; MWU)</td>
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<tr>
<td>100 pA</td>
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<td>2/8</td>
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<tr>
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<td>4.5 [0, 16]</td>
<td></td>
<td>p = 0.0847 (APs in saline vs CNO at 300 pA; MWU)</td>
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<tr>
<td>200 pA</td>
<td>A2A/saline</td>
<td>2/8</td>
<td>28 [18, 29.5]</td>
<td></td>
<td>p = 0.3821 (APs in saline vs CNO at 400 pA; MWU)</td>
</tr>
<tr>
<td></td>
<td>A2A/CNO</td>
<td>2/9</td>
<td>29 [15, 33]</td>
<td></td>
<td>p = 0.8341 (APs in saline vs CNO at 500 pA; MWU)</td>
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<td>300 pA</td>
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<td>2/8</td>
<td>15.5 [10.5, 19.5]</td>
<td></td>
<td>p = 0.0847 (APs in saline vs CNO at 300 pA; MWU)</td>
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<td>28 [18, 29.5]</td>
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<td>p = 0.8341 (APs in saline vs CNO at 500 pA; MWU)</td>
</tr>
<tr>
<td></td>
<td>A2A/CNO</td>
<td>2/9</td>
<td>29 [15, 33]</td>
<td></td>
<td>p = 0.3821 (APs in saline vs CNO at 400 pA; MWU)</td>
</tr>
<tr>
<td>500 pA</td>
<td>A2A/saline</td>
<td>2/8</td>
<td>35 [24, 36]</td>
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<td>p = 0.8341 (APs in saline vs CNO at 500 pA; MWU)</td>
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<tr>
<td></td>
<td>A2A/CNO</td>
<td>2/9</td>
<td>31 [30, 38]</td>
<td></td>
<td>p = 0.8341 (APs in saline vs CNO at 500 pA; MWU)</td>
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<th>GPe mIPSC amplitude (pA)</th>
<th>Condition</th>
<th>Treatment group</th>
<th>n (mice/cells)</th>
<th>Median [IQ range]</th>
<th>p value (comparison; test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>CNO</td>
<td>A2A</td>
<td>3/6</td>
<td>35.2 [32, 41.5]</td>
<td>p = 0.6171 (mIPSC amplitude in saline vs CNO; MWU)</td>
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<td>CNO</td>
<td>Saline</td>
<td>A2A</td>
<td>3/6</td>
<td>34.1 [27, 37.3]</td>
<td>p = 0.0082 (mIPSC frequency in saline vs CNO; MWU)</td>
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<th>GPe single unit activity</th>
<th>Condition</th>
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<th>p value (comparison; test)</th>
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<td>Baseline</td>
<td>A2A</td>
<td>4/28</td>
<td>19.4 [13, 28.5]</td>
<td></td>
<td>p = 0.0005 (baseline vs CNO; WSR)</td>
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<tr>
<td>CNO</td>
<td>A2A</td>
<td>4/28</td>
<td>14.8 [7, 24.1]</td>
<td></td>
<td>p = 0.0005 (baseline vs CNO; WSR)</td>
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<table>
<thead>
<tr>
<th>Distance traveled (m)</th>
<th>Condition</th>
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<th>n (mice/cells)</th>
<th>Median [IQ range]</th>
<th>p value (comparison; test)</th>
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</thead>
<tbody>
<tr>
<td>Baseline d1</td>
<td>A2A/vehicle</td>
<td>6</td>
<td>12 [9, 13]</td>
<td></td>
<td>p = 0.0360 (d1 baseline vs CNO; WSR)</td>
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<td>Vehicle d1</td>
<td>A2A/vehicle</td>
<td>6</td>
<td>6.6 [3.4, 8.4]</td>
<td></td>
<td>p = 0.0360 (d1 baseline vs vehicle; WSR)</td>
</tr>
<tr>
<td>CNO d1</td>
<td>A2A/vehicle</td>
<td>6</td>
<td>1.8 [0.4, 3.4]</td>
<td></td>
<td>p = 0.0360 (d1 vehicle vs CNO; WSR)</td>
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<td>Baseline d2</td>
<td>A2A/vehicle</td>
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<td>3.9 [1.7, 7.8]</td>
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<td>p = 0.0360 (d2 baseline vs vehicle; WSR)</td>
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<tr>
<td>Vehicle d2</td>
<td>A2A/vehicle</td>
<td>6</td>
<td>0 [0, 0.1]</td>
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<td>p = 0.0051 (d2 vehicle vs CNO; MWU)</td>
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<tr>
<td>CNO d2</td>
<td>A2A/vehicle</td>
<td>6</td>
<td>4.2 [3.7, 4.7]</td>
<td></td>
<td>p = 0.0048 (d3 vehicle vs CNO; MWU)</td>
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</table>

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<thead>
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<th>Distance traveled (m)</th>
<th>Condition</th>
<th>Treatment group</th>
<th>n (mice/cells)</th>
<th>Median [IQ range]</th>
<th>p value (comparison; test)</th>
</tr>
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<td>Vehicle d3</td>
<td>A2A/vehicle</td>
<td>6</td>
<td>4.2 [3.7, 4.7]</td>
<td></td>
<td>p = 0.0048 (d3 vehicle vs CNO; MWU)</td>
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Chronic D2-SPN activation downregulates autonomous STN activity

The impact of chronic chemogenetic activation of D2-SPNs in vivo on autonomous STN activity was assessed next. As predicted, CNO injection (1 mg/kg) in 1-copy adora2a-rM3Ds mice approximately every 12 hours for 3 days resulted in a reduction in the frequency, regularity, and incidence of autonomous STN activity compared with vehicle-injected littermates (Figure 4.1.2a-d; Table 4.1.2). These results indicate that elevating D2-SPN output is sufficient to produce a disruption of autonomous STN firing that appears to phenocopy Parkinsonian activity in the 6-OHDA and MitoPark models.

If the downregulation of intrinsic STN firing in PD mice is due to their disinhibition by elevated D2-SPN activity in vivo, then dopamine depletion would be expected to occlude the effect of chemogenetically activating D2-SPNs in adora2a-rM3Ds mice. To test this hypothesis, adora2a-rM3Ds mice received unilateral MFB injections of either 6-OHDA or vehicle. 2-3 weeks later, these PD and control mice received SC injections of either vehicle or CNO as described above, and the autonomous activity of STN neurons from the hemisphere ipsilateral to the MFB injection was surveyed. In concordance with our hypothesis, the frequency and incidence of autonomous firing was similarly downregulated in both of the MFB 6-OHDA groups that received SC vehicle or CNO injections, as well as in the MFB vehicle group that underwent chronic D2-SPN activation by CNO, when compared with controls that received vehicle injections in both the MFB and subcutaneously (Figure 4.1.2e; Table 4.1.2). These experiments show that 1) the impact of elevated D2-SPN activity on autonomous STN firing following dopamine depletion appears to be functionally maximized in our toxin model and therefore occludes further disruption by chemogenetic activation of the indirect pathway, and 2) a comparable level of downregulation is achieved by dopamine depletion or chemogenetic activation alone, indicating that elevated D2-SPN activity is likely responsible for triggering autonomous STN activity disruption in PD mice.
To control for possible off-target effects of the ligand CNO, C57BL/6 mice underwent the same CNO treatment paradigm as the adora2a-rM3Ds animals. When compared with intrinsic activity from control mice (Table 3.1.1), STN neurons from the CNO-injected C57BL/6 cohort were unchanged in their frequency, CV, and the percent of the population that was active relative to controls (Figure 4.1.2f, g; Table 4.1.2). These data suggest that CNO alone does not elicit changes to autonomous STN activity (e.g. through endogenous actions of CNO and/or its metabolites), but chronic activation of adora2a-rM3Ds for three days was sufficient to phenocopy the firing disruption produced by dopamine depletion with 6-OHDA.
Figure 4.1.2 Chronic chemogenetic activation of D2-SPNs disrupts autonomous STN activity. (a) Schedule of SC CNO or vehicle injection and ex vivo electrophysiological recording in adora2a-rM3Ds mice. (b) Expression of rM3D(Gs)-mCherry (red) in a subset of NeuN-immunoreactive striatal neurons (green) in an adora2a-rM3Ds mouse (arrows: expressing;
asterisks: non-expressing). (c, d) The autonomous activity of STN neurons was disrupted in slices from CNO-compared to vehicle-treated adora2a-rM3Ds mice (c, example traces; d, population data). (e, f) Consistent with Figure 3.1, the autonomous firing of STN neurons from MFB 6-OHDA-injected adora2a-rM3Ds mice that received SC vehicle injections was downregulated relative to those that received MFB and SC vehicle injections. Chemogenetic activation of D2-SPNs in vivo relatively downregulated autonomous activity in slices from vehicle- but not 6-OHDA-injected mice, indicating that in 6-OHDA-injected mice, elevated D2-SPN activity following dopamine depletion occluded the effect of CNO. Additionally, the effect of MFB 6-OHDA injection (with SC vehicle) was similar to that observed in MFB vehicle-injected, CNO-treated mice (e, example traces; f, population data). (g, h) Chronic CNO administration did not impact autonomous STN activity in otherwise naïve C57BL/6 mice (g, example traces; h, population data). * p < 0.05. n.s., not significant.
Table 4.1.2 Chronic chemogenetic activation of D2-SPNs disrupts autonomous STN activity (relates to Figure 4.1.2).

<table>
<thead>
<tr>
<th>Value</th>
<th>Treatment group</th>
<th>n (mice/cells)</th>
<th>median [IQ range]</th>
<th>p value (comparison; test)</th>
<th>Holm p value</th>
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<td>Frequency (Hz)</td>
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<td>A2A/CNO</td>
<td>3/67</td>
<td>0 [0, 3.0]</td>
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</tr>
<tr>
<td>CV</td>
<td>A2A/vehicle</td>
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<td>0.16 [0.10, 0.39]</td>
<td>p = 0.0038 (veh vs CNO; MWU)</td>
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<tr>
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<td>A2A/CNO</td>
<td>3/29</td>
<td>0.45 [0.16, 1.1]</td>
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<tr>
<td>% Active</td>
<td>A2A/vehicle</td>
<td>3/67</td>
<td>94%</td>
<td>p &lt; 0.0001 (veh vs CNO; Fisher's)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A2A/CNO</td>
<td>3/67</td>
<td>43%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>A2A MFB veh/SC veh</td>
<td>3/60</td>
<td>7.3 [0.33, 13.7]</td>
<td>p &lt; 0.0001 (veh/veh vs veh/CNO; MWU)</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>A2A MFB veh/SC CNO</td>
<td>3/58</td>
<td>0.61 [0, 5.1]</td>
<td>p = 0.0024 (veh/veh vs 6-OHDA/veh; MWU)</td>
<td>p&lt;0.0048</td>
</tr>
<tr>
<td></td>
<td>A2A MFB 6-OHDA/SC veh</td>
<td>3/61</td>
<td>3.5 [0, 7.8]</td>
<td>p = 0.50 (6-OHDA/veh vs 6-OHDA/CNO; MWU)</td>
<td>p=0.50</td>
</tr>
<tr>
<td></td>
<td>A2A MFB 6-OHDA/SC CNO</td>
<td>3/60</td>
<td>0.60 [0, 7.4]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>A2A MFB veh/SC veh</td>
<td>3/47</td>
<td>0.27 [0.15, 0.50]</td>
<td>p = 0.0003 (veh/veh vs veh/CNO; MWU)</td>
<td>p&lt;0.0010</td>
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<tr>
<td></td>
<td>A2A MFB veh/SC CNO</td>
<td>3/33</td>
<td>0.70 [0.34, 1.3]</td>
<td>p = 0.42 (veh/veh vs 6-OHDA/veh; MWU)</td>
<td>p=0.85</td>
</tr>
<tr>
<td></td>
<td>A2A MFB 6-OHDA/SC veh</td>
<td>3/37</td>
<td>0.34 [0.17, 1.0]</td>
<td>p = 0.95 (6-OHDA/veh vs 6-OHDA/CNO; MWU)</td>
<td>p=0.95</td>
</tr>
<tr>
<td></td>
<td>A2A MFB 6-OHDA/SC CNO</td>
<td>3/33</td>
<td>0.38 [0.15, 0.94]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Active</td>
<td>A2A MFB veh/SC veh</td>
<td>3/60</td>
<td>80%</td>
<td>p = 0.0012 (veh/veh vs veh/CNO; MWU)</td>
<td>p&lt;0.0036</td>
</tr>
<tr>
<td></td>
<td>A2A MFB veh/SC CNO</td>
<td>3/58</td>
<td>58%</td>
<td>p = 0.0050 (veh/veh vs 6-OHDA/veh; MWU)</td>
<td>p=0.01</td>
</tr>
<tr>
<td></td>
<td>A2A MFB 6-OHDA/SC veh</td>
<td>3/61</td>
<td>61%</td>
<td>p = 0.47 (6-OHDA/veh vs 6-OHDA/CNO; MWU)</td>
<td>p=0.47</td>
</tr>
<tr>
<td></td>
<td>A2A MFB 6-OHDA/SC CNO</td>
<td>3/60</td>
<td>55%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>C57/CNO</td>
<td>2/37</td>
<td>7.8 [0.9, 11.6]</td>
<td>p = 0.22 (cntl vs CNO; MWU)</td>
<td>p&gt;0.66</td>
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<tr>
<td>CV</td>
<td>C57/CNO</td>
<td>2/28</td>
<td>0.24 [0.16, 0.35]</td>
<td>p = 0.38 (cntl vs CNO; MWU)</td>
<td>p&gt;0.75</td>
</tr>
<tr>
<td>% Active</td>
<td>C57/CNO</td>
<td>2/37</td>
<td>78%</td>
<td>p &gt; 0.9999 (cntl vs CNO; Fisher's)</td>
<td>p=1</td>
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</table>
4.2 NMDARs in a disinhibited STN

4.2 Introduction

The origins of abnormally synchronous oscillations in the Parkinsonian brain are unclear, but the elevated correlation between cortical and STN activity is strongly associated with motor dysfunction in both experimental and idiopathic PD. Furthermore, disrupting this correlation, e.g. with STN DBS, L-DOPA, or pharmacological STN inhibition or ablation is therapeutic (Levy et al., 2001; Benabid et al., 2009; Gatev et al., 2006; Kühn et al., 2009; López-Azcárate et al., 2010). Having established that chronically activating D2-SPNs triggers the disruption of decorrelating autonomous STN firing, it may be that this emerging disinhibition of the STN and consequent rise in excitatory tone can engage cell-autonomous adaptive mechanisms to temper its excitability. In light of findings by Chu et al. (2015, 2017) demonstrating NMDAR-mediated synaptic plasticity within the STN and at its synapses in GPe following dopamine loss, we investigated the involvement of STN NMDARs in the disruption of autonomous firing in the structure.

The STN receives robust glutamatergic input mediated by AMPA and NMDARs from cortex (Afsharpour 1985a). It is also excited by parafascicular thalamus (Feger et al., 1994; Sugimoto et al. 1983; Mouroux and Feger 1993), and to a lesser extent the pedunculopontine nucleus (PPN) and superior colliculus (Carpenter et al., 1981). While AMPAR-mediated currents are dependent only on ligand binding for activation, NMDARs require strong postsynaptic depolarization in order to relieve receptors from Mg$^{2+}$ blockade and allow flow of cations through the pore (Nowak et al., 1984). It follows that, in a brain where the indirect pathway is hyperactive and disinhibiting the STN, NMDAR activation at glutamatergic inputs is more likely to occur. As NMDARs engage countless pathways that affect cellular and synaptic properties (some already demonstrated in
this structure and model in Chu et al., 2015 and 2017), they are prime candidates for instigating the alterations of intrinsic STN firing triggered by dopamine depletion and its consequent changes in circuit activity.

In addition to two obligatory GluN1 subunits, NMDARs of the STN contain GluN2B and 2D subunits according to work by Swanger et al. in the rat (2015). NMDARs are expressed largely on dendrites in the STN, but a small fraction is also found at axon terminals, putatively at GABAergic GP synapses (which fits with Chu et al.’s 2015 observations of NMDAR-mediated heterosynaptic plasticity there; Bevan et al., 1995). Upon co-activation by its ligands glutamate and glycine (or D-serine), plus sufficient postsynaptic depolarization to remove the Mg$^{2+}$ block in its pore, NMDARs can effect large local increases in $[\text{Ca}^{2+}]$ of over 5 µM (Rajdev and Reynolds 1993; Hyrc et al., 1997), due in part to the high conductance (20-50 pS; Carafoli et al., 2001) and long timescale of activation following ligand binding (EPSC decay time constant = 50-250 ms; Jonas 1993; Chu et al., 2015). Ca$^{2+}$ entering neurons through NMDARs serves as a potent and diverse second messenger that can activate ion channels, trigger synaptic plasticity, stimulate cell survival or death pathways, alter gene expression, and promote protein phosphorylation/dephosphorylation and free radical production (reviewed in Carafoli et al., 2001; Ca$^{2+}$ signaling pathways discussed further in Section 5.1). The engagement of these different signaling pathways depends on several factors, including 1) the timing and strength of NMDAR activation; 2) intracellular localization of proteins and organelles relative to the NMDARs; 3) the metabolic state of the cell at the time of activation; and 4) the location of NMDARs relative to the active zone of the synapse. With the plethora of confirmed and potential signaling pathways and plasticities downstream, we investigated the involvement of STN NMDARs in the development of PD pathology using pharmacology and a genetic knockdown technique.
4.2 Experimental Approach

Animal preparation

C57BL/6 males that received intracranial injections of either vehicle or 6-OHDA in the MFB were used to test the effect of NMDAR activation on STN neurons in slice, and whether that effect was occluded by plasticity induced in the 6-OHDA PD model. Furthermore, to determine whether STN NMDARs were necessary for the disruption of autonomous STN activity in the 6-OHDA mouse, adult male Grin1 Doylex/lox animals received stereotaxic injections of 1) either 6-OHDA or vehicle in the MFB and 2) AAV expressing either cre-eGFP or eGFP alone to knock down NMDARs and control for viral expression, respectively.

Electrophysiological recordings

To assess the effect of chronic NMDA exposure on STN activity, electrophysiological slices from vehicle- or 6-OHDA-injected C57BL/6 mice were divided into two groups: half of the slices from each animal were incubated in a holding chamber containing 25 µM NMDA in ACSF for 1 hour prior to recording, while the other half remained in control ACSF for the duration. Autonomous activity was surveyed and compared in NMDA-incubated and control tissue. Electrophysiological slices were similarly prepared from Grin1 Doylex/lox animals and surveyed for autonomous STN firing 2-3 weeks after stereotaxic injections.

4.2 Results

NMDAR activation ex vivo is sufficient to disrupt autonomous STN activity

To determine the effect of ex vivo NMDAR activation on autonomous STN activity, cell-attached recordings in the presence of synaptic blockers were compared between slices from MFB vehicle-injected mice that were incubated in either 25 µM NMDA or control ACSF for an hour prior to the
activity survey. Replicating the results of Atherton et al. (2016) performed in surgically naïve C57BL/6 animals, autonomous STN activity in MFB vehicle-injected tissue was downregulated in slices that were exposed to NMDA compared with control slices from the same preparations (Figure 4.2.1a, b; Table 4.2.1). If activation of NMDARs in vivo is responsible for the downregulation of autonomous STN activity in 6-OHDA-injected mice, the effect of NMDAR activation ex vivo on autonomous STN activity seen in MFB vehicle-injected mice should be occluded in slices from 6-OHDA-injected mice. Consistent with this hypothesis, we found that NMDAR activation ex vivo downregulated autonomous STN activity in slices from vehicle-but not 6-OHDA-injected mice relative to untreated slices (Figure 4.2.1a, b; Table 4.2.1). These results indicate that ex vivo activation of STN NMDARs can replicate the PD-like disruption of autonomous firing, and that this effect is occluded by the in vivo plasticity following dopamine depletion.

**STN NMDARs are necessary to disrupt autonomous STN activity in PD mice**

To determine whether STN NMDAR activation in vivo is driving autonomous activity downregulation in 6-OHDA-injected animals, we used a knockdown model to eliminate STN NMDAR expression in PD mice. The rates of autonomous STN activity were compared between groups of Grin1lox/lox mice in which STN NMDARs were knocked out (via the cre-lox system flanking the GluN1 gene and the viral expression of cre-recombinase, ), or a control viral injection was made. As in C57BL/6 mice, 6-OHDA injection into the MFB of Grin1lox/lox mice (with eGFP expressed in STN and NMDARs intact) capitulated the disruption of autonomous STN firing compared with MFB vehicle-injected controls (Figure 4.2.1c-f; Table 4.2.1). In agreement with the hypothesis that this activity disruption is engaged by NMDAR activation, PD mice with STN NMDARs knocked out (cre-eGFP-expressing) preserved their intrinsic firing, while those expressing eGFP exhibited the predicted slow, irregular firing and reduction in the proportion of
active neurons (Figure 4.2.1c-f; Table 4.2.1). These results support the theory that autonomous STN activity disruption is triggered by excessive NMDAR activation following dopamine depletion and elevated D2-SPN output.
**Figure 4.2.1** STN NMDAR activation triggers STN firing disruption. (a, b) Consistent with the results in **Figure 3.1.1**, the autonomous firing of STN neurons in untreated slices from 6-OHDA-injected mice was downregulated relative to that in untreated slices from vehicle-injected mice. Activation of STN NMDARs *ex vivo* with 25 µM NMDA for 1 hour downregulated autonomous activity in slices from vehicle- but not 6-OHDA-injected mice, arguing that in 6-OHDA-injected mouse NMDAR activation *in vivo* occluded the downregulatory effect of NMDAR activation *ex vivo* (a, examples; b, population data). (c, d) Confocal micrographs of viral-mediated eGFP (c, green) or cre-eGFP (d, green) expression in NeuN-immunoreactive (red) STN neurons (arrows). (e, f) In eGFP-expressing, NMDAR-intact STN neurons, autonomous activity was downregulated in brain slices from 6-OHDA-injected mice relative to activity in vehicle-injected mice. However, in cre-eGFP-expressing, NMDAR-knockdown STN neurons, downregulation of autonomous activity in 6-OHDA-injected mice was prevented (e, examples; f, population data). * p < 0.05. n.s., not significant.
Table 4.2.1 STN NMDAR activation triggers STN firing disruption (relates to Figure 4.2.1).

<table>
<thead>
<tr>
<th>Value</th>
<th>Treatment Group</th>
<th>Condition</th>
<th>n (mice/cells)</th>
<th>Median [IQ range]</th>
<th>p value (comparison; test)</th>
<th>Holm p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (Hz)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>Control</td>
<td>6/60</td>
<td>8.3 [2.4, 12.9]</td>
<td>p &lt; 0.0001 (veh/cntl vs veh/NMDA; MWU)</td>
<td>p_h &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>NMDA</td>
<td>Control</td>
<td>6/60</td>
<td>0.28 [0, 48]</td>
<td>p &lt; 0.0001 (veh/cntl vs 6-OHDA/cntl; MWU)</td>
<td>p_h &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
<td>Control</td>
<td>6/60</td>
<td>0.94 [0, 4.6]</td>
<td>p = 0.48 (6-OHDA/cntl vs 6-OHDA/NMDA; MWU)</td>
<td>p_h = 0.48</td>
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<tr>
<td>CV</td>
<td>Vehicle</td>
<td>Control</td>
<td>6/51</td>
<td>0.25 [0.15, 0.56]</td>
<td>p = 0.021 (veh/cntl vs veh/NMDA; MWU)</td>
<td>p_h = 0.042</td>
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<tr>
<td></td>
<td>NMDA</td>
<td>Control</td>
<td>6/32</td>
<td>0.68 [0.18, 1.1]</td>
<td>p = 0.0010 (veh/cntl vs 6-OHDA/cntl; MWU)</td>
<td>p_h = 0.03</td>
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<td></td>
<td>6-OHDA</td>
<td>Control</td>
<td>6/35</td>
<td>0.37 [0.19, 1.1]</td>
<td>p = 0.61 (6-OHDA/cntl vs 6-OHDA/NMDA; MWU)</td>
<td>p_h = 0.61</td>
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<tr>
<td>% Active</td>
<td>Vehicle</td>
<td>Control</td>
<td>6/60</td>
<td>85%</td>
<td>p &lt; 0.0001 (veh/cntl vs veh/NMDA; Fisher's)</td>
<td>p_h &lt; 0.0001</td>
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<td>NMDA</td>
<td>Control</td>
<td>6/60</td>
<td>57%</td>
<td>p &lt; 0.0001 (veh/cntl vs 6-OHDA/cntl; Fisher's)</td>
<td>p_h &lt; 0.0001</td>
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<td>6-OHDA</td>
<td>Control</td>
<td>6/60</td>
<td>58%</td>
<td>p = 0.24 (6-OHDA/cntl vs 6-OHDA/NMDA; Fisher's)</td>
<td>p_h = 0.24</td>
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<td>Frequency (Hz)</td>
<td>Vehicle + eGFP</td>
<td>3/84</td>
<td>14.3 [8.5, 23.2]</td>
<td>p &lt; 0.0001 (veh/eGFP vs 6-OHDA/eGFP; MWU)</td>
<td>p_h &lt; 0.0001</td>
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<td></td>
<td>6-OHDA + eGFP</td>
<td>3/85</td>
<td>3.6 [0, 16.2]</td>
<td>p &lt; 0.0001 (6-OHDA/eGFP vs 6-OHDA/cre-eGFP; MWU)</td>
<td>p_h = 0.0001</td>
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<td>6-OHDA + cre-eGFP</td>
<td>3/59</td>
<td>14.1 [6.7, 19.2]</td>
<td>p = 0.28 (veh/eGFP vs 6-OHDA/cre-eGFP; MWU)</td>
<td>p_h = 0.28</td>
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<td>CV</td>
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<td>3/81</td>
<td>0.16 [0.11, 0.32]</td>
<td>p = 0.032 (veh/eGFP vs 6-OHDA/eGFP; MWU)</td>
<td>p_h = 0.096</td>
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<td>6-OHDA + eGFP</td>
<td>3/53</td>
<td>0.24 [0.13, 0.58]</td>
<td>p = 0.056 (6-OHDA/eGFP vs 6-OHDA/cre-eGFP; MWU)</td>
<td>p_h = 0.11</td>
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</tr>
<tr>
<td></td>
<td>6-OHDA + cre-eGFP</td>
<td>3/54</td>
<td>0.16 [0.10, 0.55]</td>
<td>p = 0.77 (veh/eGFP vs 6-OHDA/cre-eGFP; MWU)</td>
<td>p_h = 0.77</td>
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</tr>
<tr>
<td>% Active</td>
<td>Vehicle + eGFP</td>
<td>3/84</td>
<td>96%</td>
<td>p &lt; 0.0001 (veh/eGFP vs 6-OHDA/eGFP; Fisher's)</td>
<td>p_h &lt; 0.0001</td>
<td></td>
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<tr>
<td></td>
<td>6-OHDA + eGFP</td>
<td>3/85</td>
<td>65%</td>
<td>p &lt; 0.0001 (6-OHDA/eGFP vs 6-OHDA/cre-eGFP; Fisher's)</td>
<td>p_h &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-OHDA + cre-eGFP</td>
<td>3/59</td>
<td>92%</td>
<td>p = 0.32 (veh/eGFP vs 6-OHDA/cre-eGFP; Fisher's)</td>
<td>p_h = 0.32</td>
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</table>
4.3 Conclusions

The results of Chu et al. (2017) demonstrate that chemogenetic activation in adora2a-rM3Ds mice augments the frequency (but not amplitude) of mIPSCs in the GPe ex vivo and reduces GPe activity (and locomotion) in vivo, in line with our assertions that this model is suited to reproducing Parkinsonian indirect pathway hyperactivity. Our data showing autonomous firing disruption in the STN of chemogenetically activated adora2a-rM3Ds mice support the hypothesis that elevated D2-SPN inhibition reduces activity in the GPe and triggers ultimately maladaptive changes in the STN, putatively in response to its disinhibition. The findings of Section 4.2 also argue that the cellular signaling and plasticity that follows dopamine depleting lesion to the MFB requires STN NMDARs to produce the disruption of autonomous STN activity that we have mimicked through chronic adora2a-rM3Ds activation. These data support our hypothesis that in PD, disinhibited STN neurons are subject to elevated NMDAR activation, which triggers cellular plasticity to downregulate intrinsic firing.

Given that cortico-basal ganglia-thalamo-cortical circuit activity perturbations take days or weeks to develop in the 6-OHDA rodent model of PD (e.g. Vila et al., 2000; Mallet et al., 2008a), yet nigrostriatal terminal degeneration takes place ~18-48 h following injection (Hökfelt and Ungerstedt, 1973), it follows that these late-emerging changes in neuronal activity in the PD brain likely result from compensatory and homeostatic mechanisms that follow loss of dopamine rather than dopamine loss itself. That is, when SNc terminals in the striatum begin to degenerate, the target neurons undergo plasticity to adjust to their new dopamine-deficient environment, engaging mechanisms that perhaps normalize their behavior or maximize their survival. However, what is conceivably optimal for a given neuron may not be functional in the context of
its circuit connections—that is to say, the neuron’s plasticity mechanisms to promote its individual homeostasis and survival can be ultimately maladaptive for the organism.

We have now observed in the PD mouse 3 forms of maladaptive plasticity involving STN NMDARs: 1) heterosynaptic plasticity at the GPe-STN synapse, at which excessive glutamatergic cortical input drives the potentiation of the GABAergic GPe input (Chu et al., 2015); 2) downregulation of cortico-STN synapses, which further skews STN input to its inhibitory GPe contributions (Chu et al., 2017); and 3) downregulation of autonomous STN activity (Figure 4.2.1). Important to also consider is the fact that timing, not just the overall rate of circuit activity can contribute profoundly to the way these nuclei respond to changing inputs. Key to the cortico-basal ganglia-thalamo-cortical loop in PD is the predominance of global, abnormally synchronous oscillations that develop in the absence of dopamine (Bergman and Deuschl, 2002; Gatev et al., 2006; Galvan and Wichmann, 2008; Mallet et al., 2008a, b). The parallel connectivity of the circuit (discussed in Section 1.1) puts the STN in a node at which oscillating cortical input alternately drives the excitation (via cortico-STN synapses) and inhibition (via phase-offset D2-SPN-GPe disinhibition during down-states). This means cortical input to the STN coincides with a release from GPe inhibition, making the STN more susceptible to excitation. It remains unclear the origins of pathological oscillations in PD, but in vivo recordings reveal the propensity of the Parkinsonian circuit elements to resonate synchronously—a difference Brazhnik et al. (2014) even observed in the exaggerated SNr LFP power in lesioned over non-lesioned hemispheres within 6-OHDA rats (see Section 1.2 for more on Parkinsonian circuit activity). Yet to be elucidated is the timecourse on which these plastic events occur (synchronous oscillations, autonomous STN activity loss, downregulation of cortico-STN synapses?) and which contribute to the advent of others. More progressive models (e.g. MitoPark) would provide a better opportunity to study the interactions of various plasticities during dopamine neuron degeneration, which may help
identify additional intervention targets and windows, as well any lynchpin among the observed pathologies that triggers others.
CHAPTER 5: EFFECTORS OF CELLULAR PLASTICITY IN THE PARKINSONIAN STN

5.1 Mitochondrial oxidation

5.1 Introduction

Having established that 1) in vivo STN NMDAR activation is necessary to disrupt autonomous activity in PD mice, 2) ex vivo NMDAR activation in vehicle-injected tissue is sufficient to reproduce the disruption in the STN, and 3) the effect of NMDAR activation on STN firing ex vivo is occluded by 6-OHDA-induced hemiparkinsonism, the cellular mechanisms downstream of NMDAR activation that could contribute to the chronic disruption of autonomous firing were investigated in the STN.

Our hypothesis that disinhibited activation of STN NMDARs following dopamine depletion capitulates development of the firing phenotype implies a greater-than-normal net conductance of Ca\(^{2+}\) through NMDARs. As discussed in Section 4.2, NMDARs can conduct an enormous amount of Ca\(^{2+}\) down its 10,000-fold gradient into neurons. Once inside the cell, Ca\(^{2+}\) is regulated by the endoplasmic reticulum (ER) and mitochondria, which take up, transport, and release it (Friel, 2000; Verkhratsky, 2005). Total internal cytosolic Ca\(^{2+}\) is ~1mM, but >99.9% is protein-bound in the cytosol or sequestered in the ER, leaving ~100nM free at baseline and ~1µM during stimulation (though local increases can be much higher). Inside the mitochondria, total and free Ca\(^{2+}\) is ~100µM and 100nM, taken up by the Ca\(^{2+}\)-sensitive uniporter and released by the Na\(^{+}/Ca\(^{2+}\)
Periodic, physiological levels of Ca\(^{2+}\) entry into the mitochondrial matrix following excitation stimulates ATP and NADH production by activating ATP synthase and enzymes of the Kreb’s cycle (Denton 2009). In contrast, excessive Ca\(^{2+}\) entry specifically through NMDARs can lead to necrotic cell death (wherein the dying cell’s contents are simply released into the surrounding tissue upon membrane rupture, leading to an inflammatory response and collateral damage to neighboring cells), while moderate excitotoxicity initiates apoptotic pathways (in which cells undergo programmed cell death and debris can be safely removed by phagocytes; NMDAR-activated cell death pathways reviewed by Nicholls and Bud, 1998). However, before the threshold to initiate cell death is reached, elevated Ca\(^{2+}\) influx through NMDARs can induce a host of adaptations to resist excitotoxicity and re-equilibrate to the cell’s new environment. For example, in Chu et al., (2017), we found a reduction in the number of cortico-STN synapses (per vesicular glutamate transporter-1 (vGluT1) immunoreactivity) following chronic disinhibition of the STN by either dopamine depletion or adora2a-rM3Ds activation (as described in Sections 3.1 and 4.1 respectively). In alignment with the results in Section 4.2, this apparent adaptation to disinhibited STN activity in PD mice required NMDAR activation, as its knockout in the STN blocked the reduction of vGlut1 immunoreactivity. These studies exemplify the broad range of NMDAR-mediated cellular plasticity in response to changing stimulation in the STN.

**Mitochondria, calcium, and ROS**

In a stimulated cell, the cytosolic free [Ca\(^{2+}\)] rises to levels 10-fold higher than in the mitochondria, triggering activation of uniporters and flooding the organelles with Ca\(^{2+}\). In this way mitochondria sequester Ca\(^{2+}\) in large quantities, which often becomes bound to free inorganic phosphate (P\(_i\);
Pivovarova and Andrews, 2010). When mitochondria take up this excess of Ca\(^{2+}\), effectively buffering the cell’s cytosolic [Ca\(^{2+}\)] and resisting necrotic cell death, oxidative phosphorylation ramps up and produces both more ATP and consequently more ROS (for a review of mitochondrial ROS production, see Lambert and Brand, 2009 or Adam-Vizi, 2005). Indeed, Ca\(^{2+}\) handling by the neuron and its organelles is extremely nuanced and capable of producing a spectrum of outcomes. Gleichmann and Mattson (2011) argue that mitochondrial buffering of calcium reduces the peak rate of O\(_2\) consumption by discounting the short-term cost of ATP production (protons pumped) to meet the immediate energetic needs of the excited cell at the expense of additional O\(_2\) consumption during metabolically stable times to extrude accumulated calcium ions. Calcium regulation by mitochondria has been proposed to both blunt the effect of subsequent depolarizations (e.g. if cytosolic [Ca\(^{2+}\)] increases are prolonged to reduce the electrochemical gradient across the cell membrane during NMDAR activation) and to increase Ca\(^{2+}\) influx into the cell by sequestering cytosolic Ca\(^{2+}\) (Nicholls, 2005). When persistent, repeated stimulation chronically elevates cytosolic [Ca\(^{2+}\)] such that mitochondrial extrusion of Ca\(^{2+}\) into the cytosol is impeded, this leads to its excessive accumulation in mitochondria. Ca\(^{2+}\) loading decreases both the membrane potential across the inner mitochondrial membrane and the hydrogen ion concentration gradient (dpH), thereby slowing or reversing ATP synthesis (Gleichmann and Mattson, 2011). Thus there exists an enormous dynamic range and functionality of Ca\(^{2+}\) regulation in mitochondria that variably impact a cell’s metabolism according to its needs and demands—stimulating ATP production, reversing it, or even initiating cell death pathways. One might predict that a neuron overloaded with Ca\(^{2+}\) would not be able to maintain the energetically costly autonomous firing that healthy STN neurons exhibit. However, it is unlikely that Ca\(^{2+}\) distribution dynamics due to ongoing NMDAR activation in vivo would persistently disrupt STN activity in a brain slice preparation, particularly in the presence of glutamate receptor
antagonists. We therefore sought signaling pathways secondary to Ca\textsuperscript{2+} entry through NMDARs that could induce enduring alterations in the cell’s autonomous firing properties.

Bindokas et al. (1996) demonstrated the Ca\textsuperscript{2+}-dependent elevation of the ROS superoxide (O\textsubscript{2}\textsuperscript{-}) following NMDAR activation using microfluorimetry of hydroethidine oxidation by O\textsubscript{2}\textsuperscript{-} in pyramidal neurons of the rat hippocampus. Indeed, oxidative phosphorylation to produce ATP (e.g. following stimulation and Ca\textsuperscript{2+} influx) generates a substantial amount of O\textsubscript{2}\textsuperscript{-} at complexes I, II, and III of the ETC depending on the respiratory substrates available. For example, high levels of succinate would be reduced to fumarate by Complex II, leading to the generation of a proton motive force and the depletion of available oxidized quinone (Q, electron carriers). This forces the reactions of Complex I to reverse, donating an electron from reduced Q (ubiquinol, QH\textsubscript{2}) to O\textsubscript{2} and releasing O\textsubscript{2}\textsuperscript{-} into the matrix (termed “reverse electron transport”; Lambert and Brand, 2009). ROS can serve as signaling molecules (e.g. by activating tyrosine kinases, MAP kinases, and Ras proteins to mediate processes like cell survival and proliferation, antioxidant gene regulation, senescence, and apoptosis), but in excess can also induce harmful oxidation of proteins and lipids and damage nuclear or mitochondrial DNA (ROS signaling reviewed by Cui et al., 2012 and by Ray et al., 2012). However, cells have several defense mechanisms in place to resist oxidative injury and engagement of cell death cascades following mitochondrial stimulation and accumulation of ROS, suggesting a role for moderate levels of ROS generated by an environmental stressor to trigger adaptations for survival (reviewed by Sena and Chandel, 2012). Indeed, the neuron is rife with mechanisms for tightly regulating ROS and evidence for their downstream signaling continues to emerge:

ROS levels can, for example, be reduced by mild uncoupling (i.e. the leaking of H\textsuperscript{+} from the intermembrane space), which diminishes their production and reduces the proton motive force while
adequately maintaining ATP synthesis (Brand 2000). Anti-oxidant systems of reductants in the mitochondria and cytosol also convert ROS into less-reactive byproducts. Thiol groups on the anti-oxidant glutathione (GSH) promote the reduction of disulfide bonds in target proteins and GSH’s concomitant oxidation to glutathione disulfide GSSG. The GSSH population is then reduced again by glutathione reductase, converting NADPH to NADP+ in the process (mitochondrial ROS dynamics reviewed in Stowe and Camara, 2009). (GSH/GSSG also serves as a measure of oxidative state when expressed with a redox-sensitive fluorescent indicator such as MTS-roGFP, utilized here in section 5.1; Hanson et al., 2004.) The heme group of cytochrome c is capable of both donating and accepting electrons depending on its valence (as it does when transferring electrons between Complexes III and IV in the ETC), but cytochrome c has also been shown to act as an anti-oxidant in its water-soluble form during reverse electron transport (described above), oxidizing O2− to O2 (Korshunov et al., 1999). Superoxide dismutase (SOD), found endogenously in the cytosol and intermembrane space of mitochondria (SOD1), in the mitochondrial matrix (SOD2), and extracellularly (SOD3), also participates in the anti-oxidant cycle by disumtating O2− into O2 or hydrogen peroxide (H2O2), which is then converted to H2O by glutathione peroxidase (2 GSH + 2 H2O2 → GSSG + 2 H2O) or peroxisome-localized catalase (2 H2O2 → 2 H2O + O2). The cell’s capacity to mitigate damage from ROS through such a diversity of proteins and reactions also provides an arsenal of discreet signaling pathways that can be engaged in response to ROS production or its correlates.

For instance, the intermediate ROS H2O2 (from the dismutation of O2− by SOD) can permeate membranes and act as a potent inter- and intracellular signaling molecule and neuromodulator before it’s broken down. HEK 293 cells respond to 5 mM H2O2 by depolarizing due to Na+ and Ca2+ influx as the ROS directly activates transient receptor potential (TRP) channels (Wehage et al., 2002). Smith et al. (2003) showed that 10 mM H2O2 induces a cation current that leads to
Ca\textsuperscript{2+} overload and toxicity in rat striatal neurons. Conversely, in neurons of the nucleus tractus solitarii (nTS), 500 µM H\textsubscript{2}O\textsubscript{2} triggered an acute decrease in input resistance, hyperpolarization of the resting membrane potential and AP threshold, and reduction in excitability recorded in rat brainstem slices—likely due to increased conductance of inwardly-rectifying (IR) K\textsuperscript{+} channels (Ostrowski et al., 2014). Indeed, a review of H\textsubscript{2}O\textsubscript{2} neuromodulation by M. Rice (2011) implicated the IR K\textsubscript{ATP} channel as a common effector of rapid H\textsubscript{2}O\textsubscript{2} modulation in the nigrostriatal dopamine system (e.g. Lee et al., 2015, in which they showed both H\textsubscript{2}O\textsubscript{2}-mediated inhibition of DA neurons via K\textsubscript{ATP} channels and enhanced excitability of GABAergic SPNs and SNr neurons via TRPM2 in guinea pig slices). In Bao et al. (2009), the Rice group also suggested that the primary source of H\textsubscript{2}O\textsubscript{2} in striatal neurons following dopamine stimulation was the mitochondria (not MAO or NADPH oxidase (Nox)). They demonstrate that complex I inhibition in the presence of the complex II substrate succinate abolished both the detection of H\textsubscript{2}O\textsubscript{2} and its physiological effects in the slice while maintaining ATP production, but inhibition of MAOs or Nox did not change H\textsubscript{2}O\textsubscript{2} production. Tretter and Adam-Vizi (2004) suggest that the Kreb’s cycle enzyme α-ketoglutarate dehydrogenase (α-KGDH) is also stimulated by Ca\textsuperscript{2+} to produce H\textsubscript{2}O\textsubscript{2} (even more than the ETC, according to Starkov et al., 2004), providing another potential source of mitochondrial ROS.

Given our evidence that NMDAR activation in the disinhibited STN triggers firing disruption in PD, together with the body of literature connecting Ca\textsuperscript{2+} influx through NMDARs to the stimulation of mitochondrial ROS production and their downstream signaling pathways, STN neurons were assessed for their levels of mitochondrial oxidation and the impact of the ROS H\textsubscript{2}O\textsubscript{2} on autonomous activity.
5.1 Experimental Approach

Animal preparation

To measure the oxidative state of STN neurons’ mitochondria, adult male C57BL/6 mice received stereotaxic injections of 1) either 6-OHDA or vehicle in the MFB and 2) AAV expressing MTS-roGFP in the STN. Animals used in pharmacological experiments to test the effect of catalase only received the first injection. Recordings were made 2-3 weeks following surgery.

2-Photon imaging

Acute slices were made as for electrophysiology to image AAV-MTS-roGFP fluorescence as a metric of oxidation in the STN of PD and control mice. Z-stacks were taken first in control solution (containing blockers of synaptic transmission, as in recordings of autonomous activity) to record the baseline fluorescence (oxidation) of STN mitochondria in each condition. Control images were normalized to the maximum and minimum fluorescence intensities, measured in Z-stacks of the same region 10 minutes after application of each DTT (2 mM) to fully reduce and then aldrithiol (200 µM) to fully oxidize the tissue.

Electrophysiological recordings

To test the contribution of the ROS signaling molecule H$_2$O$_2$ to STN firing perturbations in PD mice, recordings of autonomous activity were made in the cell-attached configuration before and after application of catalase (250 U/mL) to break it down.
5.1 Results

*Mitochondrial ROS is elevated in STN neurons from PD mice*

To test the hypothesis that elevated mitochondrial ROS production leads to the disruption of autonomous activity, STN neurons were compared between 6-OHDA and vehicle-injected groups for 1) the relative oxidation of mitochondria, per 2PLSM imaging of the fluorescent indicator MTS-roGFP, and 2) the response of intrinsic firing to the acute breakdown of the ROS $\text{H}_2\text{O}_2$ by bath application of cell-permeable catalase (250 U/mL). Consistent with elevated ROS production following chronic dopamine depletion, STN neurons from PD mice exhibited higher relative mitochondrial oxidation compared with neurons from MFB vehicle-injected controls (Figure 5.1.1a, b; Table 5.1.1). Furthermore, acute catalase treatment rescued autonomous firing in PD slices, with relatively minimal effects on those from vehicle-injected controls, suggesting that $\text{H}_2\text{O}_2$ mediates the disruption of intrinsic activity (Figure 5.1.1c, d; Table 5.1.1).
Figure 5.1.1 STN neurons from PD mice exhibit elevated mitochondrial oxidation and H$_2$O$_2$-dependent autonomous activity disruption. (a) MTS-roGFP expression (green) in STN neurons (gray, arrows) imaged under 2-photon laser scanning fluorescent and Dodt contrast microscopy. (b) The relative oxidation of mitochondria in STN neurons was elevated in slices from 6-OHDA- relative to vehicle-treated mice. (c, d) Breakdown of H$_2$O$_2$ with catalase rescued autonomous STN activity in slices from PD mice but had relatively minimal effects on neurons from vehicle-treated mice (c, examples; d, population data). * p < 0.05.
Table 5.1.1 STN neurons from PD mice exhibit elevated mitochondrial oxidation and H$_2$O$_2$-dependent autonomous activity disruption (relates to Figure 5.1.1).

<table>
<thead>
<tr>
<th>value</th>
<th>treatment group</th>
<th>condition</th>
<th>n (mice/cells)</th>
<th>median [IQ range]</th>
<th>p value (comparison; test stat)</th>
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<tbody>
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<td>Relative Oxidation</td>
<td>vehicle control</td>
<td>4/21</td>
<td>0.30 [0.22, 0.34]</td>
<td>p = 0.0001 (veh vs 6-OHDA; MWU)</td>
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</tr>
<tr>
<td></td>
<td>6-OHDA</td>
<td>4/24</td>
<td>0.40 [0.34, 0.50]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔFrequency (Hz)</td>
<td>vehicle (+ catalase)</td>
<td>7/13</td>
<td>2.0 [1.0, 4.0]</td>
<td>p = 0.0099 (veh vs 6-OHDA Δfreq; MWU)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
<td>6/10</td>
<td>5.6 [4.0, 8.3]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔCV</td>
<td>vehicle (+ catalase)</td>
<td>7/13</td>
<td>-0.042 [-0.078, -0.0023]</td>
<td>p &lt; 0.0001 (veh vs 6-OHDA ΔCV; MWU)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
<td>6/8</td>
<td>-1.3 [-2.2, -0.32]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.2 \( \text{K}_{\text{ATP}} \) channel activation

5.2 Introduction

In STN neurons, NMDAR (and mGluR) activation can stimulate the opening of ATP-sensitive \( \text{K}^+ \) (\( \text{K}_{\text{ATP}} \)) channels, which limit activity. \( \text{K}_{\text{ATP}} \) channel opening is promoted by multiple factors, including a fall in the ratio of cytosolic ATP to ADP (Nichols, 2006), the direct effects of ROS or reactive nitrogen species (RNS) (Ichinari et al., 1996; Kawano et al., 2009; Lee et al., 2015), and neuromodulation by ROS- and RNS-linked second messenger pathways (Shen and Johnson, 2010; Zhang et al., 2014), all of which are influenced by NMDAR activation (Dugan et al., 1995; Shen and Johnson, 2010). The Rice lab asserts that \( \text{H}_2\text{O}_2 \) directly upregulates \( \text{K}_{\text{ATP}} \) channels in striatal and nigral neurons (Bao et al., 2005; Bao et al., 2009; Avshalumov et al., 2005). Indeed, our own lab showed recently that chronic STN NMDAR activation in HD mice (due to their intrinsically impaired astrocytic glutamate uptake) and in WT mice (through application of exogenous NMDA) mediates persistent downregulation of autonomous activity through \( \text{H}_2\text{O}_2 \)-dependent \( \text{K}_{\text{ATP}} \) channel opening (Atherton et al., 2016). We therefore tested whether \( \text{K}_{\text{ATP}} \) channel activation is the primary actuator of autonomous STN activity disruption in our models of PD.

The \( \text{K}_{\text{ATP}} \) channel

The \( \text{K}_{\text{ATP}} \) channel gets its name by virtue of its modulation by the cytosolic ATP:ADP ratio. Interestingly, both ATP and ADP molecules independently and oppositely influence the \( \text{K}_{\text{ATP}} \) channel, and at different subunits (Tucker et al., 1998). The sulfonylurea receptor (SUR) class of regulatory subunits (SUR1, SUR2A and SUR2B) dimerize with the inwardly rectifying potassium channel subunits (Kir)—either Kir6.1 or Kir6.2 (Clement et al 1997)—to form octomeric \( \text{K}^+ \)-selective channels comprising the four Kir/SUR heterodimers of the \( \text{K}_{\text{ATP}} \) channel (Shyng and Nichols 1997). The Kir subunits form the pore lining and are sensitive to channel agonism by
ADP, while ATP binds the sulfonylureas to inhibit the channel (Tucker et al., 1998). Neuronal $K_{\text{ATP}}$ channels are typically made up of SUR1 and Kir6.2 subunits, while cardiac and smooth muscles contain SUR2A and SUR2B, respectively (Karschin et al., 1997; Tucker et al., 1998). Glibenclamide, which binds SUR1 and SUR2 subtypes, serves as an effective inhibitor of $K_{\text{ATP}}$ channels in neurons (for a review of $K_{\text{ATP}}$ channel modulation, see Gribble and Reimann, 2002). The dynamic modulation of the $K_{\text{ATP}}$ channel by ATP and ADP confers the ability to translate the cell’s metabolic state directly into a change in membrane potential. An example of metabolic signaling with $K_{\text{ATP}}$ channels is apparent in pancreatic beta cells: an increase in glucose metabolism yields more cellular ATP (and relatively less ADP), thereby closing the $K_{\text{ATP}}$ channel, depolarizing the cell, activating voltage-gated L-type Ca$^{2+}$ channels and triggering insulin secretion in response to the influx of Ca$^{2+}$ (Aguilar-Bryan and Bryan, 1999).

Given that 1) PD mice exhibit elevated mitochondrial oxidation and acute breakdown of H$_2$O$_2$ rescues firing (Section 5.1), 2) H$_2$O$_2$ can enact changes in membrane properties by modulating $K_{\text{ATP}}$ channel activity (Avshalumov et al., 2005; Bao et al., 2005; Bao et al., 2009), and 3) ex vivo NMDAR activation has been independently linked to both autonomous STN activity disruption (Section 4.2) and $K_{\text{ATP}}$ channel activation in STN neurons (Shen and Johnson, 2010; Atherton et al., 2016), a hypothesis was developed stipulating that STN NMDAR-stimulated H$_2$O$_2$ activates $K_{\text{ATP}}$ channels in PD mice, leading to hyperpolarization and a disruption of autonomous firing.
5.2 Experimental Approach

Animal preparation

Stereotactically-guided injections (Section 2.2) were made of either 6-OHDA or vehicle solution into the MFB. 2-3 weeks following surgery, 250-µm saggital brain slices were prepared for electrophysiological recording (Section 2.3).

Electrophysiological recording

To test the contribution of the $K_{ATP}$ channel to STN firing perturbations in PD mouse models, recordings of autonomous activity were made in the cell-attached configuration before and after application of the $K_{ATP}$ channel antagonist glibenclamide (100 nM).

5.2 Results

Parkinsonian autonomous STN activity is $K_{ATP}$ channel-dependent

In support of the hypothesis that activation of $K_{ATP}$ channels causes the disruption of autonomous STN activity, the $K_{ATP}$ channel inhibitor glibenclamide (100 nM) increased the frequency, regularity, and incidence of autonomous firing in both 6-OHDA-lesioned and MitoPark mice (Figure 5.2.1a-d; Table 5.2.1). $K_{ATP}$ channel inhibition similarly rescued STN firing in adora2a-rM3Ds mice in which D2-SPNs had been activated chemogenetically (as described in Section 4.1; Figure 5.2.1e, f; Table 5.2.1), lending further support to the hypothesis that elevated D2-SPN activity in DA-intact mice drives the same STN adaptations as in the experimental PD brain.
**Figure 5.2.1** K<sub>ATP</sub> channel activation underlies autonomous STN activity disruption in PD models. (a, b) Inhibition of K<sub>ATP</sub> channels with glibenclamide (100 nM) rescued autonomous STN activity in slices from 6-OHDA-injected mice but had relatively minimal effects on neurons from MFB vehicle-injected mice (a, examples; b, population data). (c, d) Glibenclamide increased the frequency and regularity of firing to a significantly greater degree in MitoPark mice than in WT littermates (c, examples; d, population data). (e, f) Glibenclamide also increased the frequency and regularity of STN firing from CNO-treated (dopamine-intact) adora2a-rM3Ds mice but had little effect on neurons from vehicle-treated adora2a-rM3Ds mice (e, examples; f, population data). * p < 0.05.
Table 5.2.1 $K_{ATP}$ channel activation underlies autonomous STN activity disruption in PD models (refers to Figure 5.2.1).

<table>
<thead>
<tr>
<th>Value</th>
<th>Treatment group</th>
<th>Condition</th>
<th>n (mice/neurons)</th>
<th>Median [IQ range]</th>
<th>p value (comparison; test stat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta$Freq (Hz)</td>
<td>vehicle (+ glibenclamide)</td>
<td>9/16</td>
<td>1.2 [0.69, 3.9]</td>
<td>p = 0.035 (veh vs 6-OHDA $\Delta$Freq; MWU)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
<td>10/16</td>
<td>6.4 [1.2, 9.2]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$CV</td>
<td>vehicle (+ glibenclamide)</td>
<td>9/16</td>
<td>-0.017 [-0.042, -0.0047]</td>
<td>p = 0.00043 (veh vs 6-OHDA $\Delta$CV; MWU)</td>
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</tr>
<tr>
<td></td>
<td>6-OHDA</td>
<td>10/10</td>
<td>-0.88 [-1.3, -0.29]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$Freq (Hz)</td>
<td>A2A/vehicle (+ glibenclamide)</td>
<td>3/10</td>
<td>0.33 [-0.71, 1.0]</td>
<td>p = 0.0030 (vehicle vs CNO; MWU)</td>
<td></td>
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<tr>
<td></td>
<td>A2A/CNO</td>
<td>3/9</td>
<td>2.4 [2.0, 3.8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$CV</td>
<td>A2A/vehicle (+ glibenclamide)</td>
<td>3/9</td>
<td>0.0090 [-0.052, 0.022]</td>
<td>p = 0.029 (vehicle vs CNO; MWU)</td>
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<tr>
<td></td>
<td>A2A/CNO</td>
<td>3/5</td>
<td>-0.20 [-0.38, -0.087]</td>
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<tr>
<td>$\Delta$Freq (Hz)</td>
<td>WT (+ glibenclamide)</td>
<td>3/7</td>
<td>6.8 [-1.0, 7.3]</td>
<td>p = 0.042 (WT vs mitoPark; MWU)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mitoPark</td>
<td>3/9</td>
<td>8.6 [5.8, 15.2]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$CV</td>
<td>WT (+ glibenclamide)</td>
<td>3/7</td>
<td>0.02 [-0.05, 0.10]</td>
<td>p = 0.0003 (WT vs mitoPark; MWU)</td>
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<tr>
<td></td>
<td>mitoPark</td>
<td>3/9</td>
<td>-0.96 [-1.3, -0.16]</td>
<td></td>
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</tbody>
</table>
As discussed in Section 5.1, ROS like H$_2$O$_2$ play an influential role in the cell’s oxidative state and related signaling; but through its relationship to the K$_{ATP}$ channel, H$_2$O$_2$ seemingly serves as a metabolic signal as well. Indeed, Ichinari et al (1996) found that H$_2$O$_2$ upregulates K$_{ATP}$ channel activity by decreasing its sensitivity to ATP, an effect that’s potentiated by ADP. Further demonstrating the modulation of K$_{ATP}$ channel activity by H$_2$O$_2$, Avshalumov et al. (2005) showed exogenous H$_2$O$_2$ or 1mM mercaptosuccinate (MCS, a GSH peroxidase inhibitor) causes K$_{ATP}$ channel-dependent hyperpolarization, as does catalase inhibition in midbrain DA neurons in guinea pig slices. In STN neurons, Atherton et al (2016) found that ex vivo application of MCS to elevate H$_2$O$_2$ evoked a disruption of autonomous firing similar to NMDA pre-treatment, both of which were K$_{ATP}$ channel dependent since antagonism with glibenclamide restored firing. The present study lends further support to the theory that H$_2$O$_2$ modulates K$_{ATP}$ channel activity, because 1) rescue of autonomous STN activity in PD mice to control levels can be achieved with either K$_{ATP}$ channel blockade or H$_2$O$_2$ breakdown, and 2) glibenclamide occludes the effect of catalase on autonomous STN firing in PD mice, suggesting the two interventions enact their changes to intrinsic activity via the same signaling pathway (Fig. 5.2.2a-c; Table 5.2.2).

Shen and Johnson (2010) report that acute (<5 min) NMDAR activation increased K$_{ATP}$ channel current in rat STN slices, which was blocked by supplementation with ATP (which negatively modulates K$_{ATP}$ activity, depolarizing cells when the metabolic substrate is abundant). The possibility that an ATP insufficiency was contributing to elevated K$_{ATP}$ channel activity was therefore tested. However, dialysis of STN neurons with 2 mM ATP did not consistently change firing rates in STN neurons from either 6-OHDA or vehicle-injected mice (Fig. 5.2.2d, e; Table 5.2.2), suggesting that the mechanism activating K$_{ATP}$ channels in PD following prolonged (>1 hr) STN NMDAR hyperactivity involves another modulatory factor.
It was recently reported that SK channels—which are fundamental to autonomous, synaptically generated, and rebound burst firing behavior in STN neurons (Hallworth et al., 2003)—are upregulated in the PD rodent STN (Mourre et al., 2017). But inhibition of SK channels with apamin (10 nM) also failed to rescue STN activity ex vivo (Fig. 5.2.2f, g; Table 5.2.2). Taken together, the data argue that in PD mice, elevated H$_2$O$_2$ production leads to increased K$_{ATP}$ channel activation, which reduces the frequency and regularity of autonomous STN activity.
**Figure 5.2.2** $K_{\text{ATP}}$ channel-dependent disruption of autonomous STN activity is mediated by $\text{H}_2\text{O}_2$ in PD mice. (a) The frequency and regularity of autonomous STN activity in tissue from 6-OHDA-treated mice is rescued by both glibenclamide and catalase to levels that are not significantly different from that in control tissue under control conditions. (b, c) Application of catalase in addition to glibenclamide does not further enhance the rescue of autonomous activity in PD mice achieved by glibenclamide alone (b, population data; c, example...
trace). (d, e) The autonomous activity of STN neurons from vehicle-injected (black) and 6-OHDA-injected (green) mice recorded in the cell-attached configuration (d, top traces) was not affected by establishment of the whole-cell configuration and dialysis with 2 mM ATP (d, bottom traces; e, population data). (f, g) Inhibition of SK channels with 10 nM apamin did not rescue autonomous firing in slices from PD mice (f, examples; g, population data). * p < 0.05. n.s., not significant.
**Table 5.2.2** $K_{\text{ATP}}$ channel-dependent disruption of autonomous STN activity is mediated by $H_2O_2$ in PD mice (refers to **Figure 5.2.2**).

<table>
<thead>
<tr>
<th>Value</th>
<th>Treatment group</th>
<th>Condition</th>
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<th>Median [IQ range]</th>
<th>$p$ value (comparison; test stat)</th>
<th>Holm $p$</th>
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<tr>
<td>Frequency</td>
<td>AMC or 6-OHDA</td>
<td>AMC</td>
<td>12/66</td>
<td>9.4 [3.4, 13.9]</td>
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<td></td>
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<td></td>
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<td>6-OHDA + glib</td>
<td>10/16</td>
<td>7.1 [2.6, 11.9]</td>
<td>$p = 0.50$ (AMC vs 6-OHDA + glib; MWU)</td>
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<td>6/10</td>
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<td>0.20 [0.14, 0.36]</td>
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<td>0.62 [0, 1.5]</td>
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<td></td>
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<tr>
<td></td>
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<td>glibenclamide</td>
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<tr>
<td></td>
<td></td>
<td>glib + cat</td>
<td>5/8</td>
<td>10.8 [0.59, 19.2]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>6-OHDA</td>
<td>control (CA)</td>
<td>3/20</td>
<td>10.9 [4.8, 18.7]</td>
<td>$p = 0.11$ (cntl vs ATP; WSR)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP (WC)</td>
<td>3/20</td>
<td>9.1 [2.9, 20.1]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>control (CA)</td>
<td>4/17</td>
<td>4.7 [0, 13.8]</td>
<td>$p = 0.22$ (cntl vs ATP; WSR)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP (WC)</td>
<td>4/17</td>
<td>2.6 [0.070, 12.7]</td>
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</tr>
<tr>
<td>CV</td>
<td>6-OHDA</td>
<td>control (CA)</td>
<td>3/15</td>
<td>0.15 [0.10, 0.24]</td>
<td>$p = 0.40$ (cntl vs ATP; WSR)</td>
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<tr>
<td></td>
<td></td>
<td>ATP (WC)</td>
<td>3/15</td>
<td>0.15 [0.10, 0.24]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>control (CA)</td>
<td>4/10</td>
<td>0.10 [0.085, 0.65]</td>
<td>$p = 0.054$ (cntl vs ATP; WSR)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP (WC)</td>
<td>4/10</td>
<td>0.21 [0.089, 0.87]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔFrequency</td>
<td>6-OHDA</td>
<td>(+ apamin)</td>
<td>3/6</td>
<td>-1.2 [-2.4, 0.18]</td>
<td>$p = 0.18$ (veh vs 6-OHDA; MWU)</td>
<td></td>
</tr>
<tr>
<td>ΔCV</td>
<td>6-OHDA</td>
<td>(+ apamin)</td>
<td>3/6</td>
<td>0.089 [-1.1, 2.1]</td>
<td>$p = 0.79$ (veh vs 6-OHDA; MWU)</td>
<td></td>
</tr>
</tbody>
</table>
5.3 Conclusions

Our results showing catalase rescue of intrinsic STN firing that is occluded by glibenclamide indicate H$_2$O$_2$ serves as an important messenger molecule, contributing to a growing body of literature appreciating the diversity of mitochondrial ROS signaling. In addition to the well-known cell death pathways signaled by ROS, they are also required for synaptic plasticity and memory, with bipolar effects which are concentration-dependent (reviewed in Kishida and Klann, 2007). For example, Kamsler and Segal (2003) demonstrated that 1 μM H$_2$O$_2$ enhanced LTP twofold in hippocampal rat slices, while higher concentrations (0.5 – 5 mM) depress EPSP magnitudes instead. It seems a similar sensitivity to ROS exists in STN neurons of the PD mouse, such that their elevated production levels (per MTS-roGFP, Figure 5.2.1) induce plasticity in the neurons’ membrane properties (e.g. H$_2$O$_2$ -mediated K$_{ATP}$ channel activation) that does not involve appreciable energetic deficits (as ATP dialysis did not rescue firing, Figure 5.2.2d, e). This result aligns with other studies demonstrating a functional, putatively adaptive signaling role for mitochondrial ROS in processes ranging from metabolic adaptation and cell proliferation to immunity and autophagy (reviewed by Ray et al., 2012 as well as Sena and Chandel 2012).

Recent studies from the Johnson lab (Shen and Johnson, 2010; Shen and Johnson, 2013; Shen et al., 2014) show the activation of K$_{ATP}$ conductances with acute NMDAR and mGluR agonism in rat STN neurons ex vivo, and demonstrate the involvement of Ca$^{2+}$, NOS, and GC signaling (which our unpublished pilot data suggest are not required in our model) as well as the ratio of ADP:ATP. However, their brief (<5 min) exposure paradigm shows clear abatement of the K$_{ATP}$ current immediately following the activation period, suggesting our results indicating persistent K$_{ATP}$ channel-mediated disruption in the hours following prolonged (1 hr) NMDAR activation mediated by mitochondrial ROS is not incompatible. Indeed, it speaks to the rich variety of finely-
tuned responses to changing cellular environments that are time- and concentration-dependent. Importantly, $K_{ATP}$ channels function throughout cells of the body to modulate membrane potential in response to metabolic demands (Nichols 2006). Their sensitivity to so many modulatory factors—ATP, ADP, $H_2O_2$, NO—suggests their versatility and importance in monitoring and adjusting the energetic processes of the cell. The $K_{ATP}$ channel provides a kind of controlled $K^+$ leak current whose conductance is throttled by various indicators of activity, metabolic state, and stress. By specifically adjusting the cell’s excitability in response to these factors, $K_{ATP}$ channels behave somewhat like a thermostat for the cell’s metabolic homeostasis, incorporating a variety of information about the current energetic supply, demands, and stressors of the cell that is translated into a change in membrane potential that moderates energy expenditure. Thus, given that the cellular plasticity we see in PD is associated with disinhibited firing and NMDAR-mediated excitation, it is no surprise that the response of STN neurons is to activate a hyperpolarizing conductance that homeostatically dampens the cell’s excitability to accommodate the elevated influx of $Ca^{2+}$. 
CHAPTER 6: TARGETING INTRINSIC STN ACTIVITY TO AMELIORATE PARKINSONIAN MOTOR DEFICITS

Abnormal STN activity is thought to contribute directly to the expression of PD symptoms, since akinesia, bradykinesia, and rigidity are linked to elevated synchronization of the STN (Gatev et al., 2006; Hammond et al., 2007; Kühn et al., 2009; Tachibana et al., 2011; Shimamoto et al., 2013; Delaville et al., 2015), and manipulations of STN activity such as lesioning (Bergman et al., 1990), pharmacological silencing (Levy et al., 2001), or STN deep brain stimulation (DBS; Benabid et al., 2009) ameliorate parkinsonian motor dysfunction. Furthermore, the loss of decorrelating intrinsic STN activity may promote synchronization, so its restoration is predicted to reduce entrainment of STN neurons to cortical input.

6.1 STN NMDAR knockdown

6.1 Introduction

In Chu et al. (2015 and 2017), we exemplified several effects of NMDAR-mediated plasticity in the STN of the 6-OHDA mouse. In addition to disrupting autonomous activity in STN neurons (Figure 4.2.1), NMDAR activation following dopamine depletion strengthens GPe-STN synapses heterosynaptically via cortical transmission (Chu et al., 2015) and drives deafferentation of the hyperdirect pathway at cortico-STN synapses (Chu et al., 2017). In each of these three
studies, NMDAR knockdown prevented the PD-associated plasticities in the STN following dopamine loss. It is unclear to what degree these changes are adaptive and help preserve circuit and motor function, or whether they are ultimately maladaptive. But preventing NMDAR-mediated STN plasticity following dopamine loss may be sufficient to ameliorate motor deficits in PD mice. Simply considering the rescue of decorrelating autonomous firing afforded by STN NMDAR-KD, one might also predict the improvement of PD symptoms associated with abnormally coherent STN activity.

6.1 Experimental Approach

Animal preparation
To test whether STN NMDAR knockdown (and the resulting preservation of autonomous STN activity) could rescue motor deficits in the 6-OHDA mouse, adult male \textit{Grin1}^{lox/lox} animals received stereotaxic injections of 1) either 6-OHDA or vehicle in the MFB and 2) AAV expressing either cre-eGFP or eGFP alone to knock down NMDARs and control for viral expression, respectively. To control for NMDAR knockdown in off-target structures, in another set of \textit{Grin1}^{lox/lox} PD mice, injections were made of AAV-cre-eGFP just dorsal to the STN, in zona incerta (ZI).

Behavioral assays
2-3 weeks following stereotaxic injections, the \textit{Grin1}^{lox/lox} mice were evaluated for features of their spontaneous locomotion in the open field upon their first exposure to the paradigm (described in Section 2.5). Following open field testing, each mouse was assessed for forelimb use asymmetry with the cylinder test (Section 2.5).
6.1 Results

Based on the findings in Section 4.2 showing that PD mice lacking STN NMDARs do not lose their autonomous activity, and the fact that intrinsic activity is decorrelating (Wilson 2013), STN NMDAR knockdown was predicted to prevent the motor deficits in PD mice associated with coherent STN activity. Indeed, hemiparkinsonian mice with intact STN NMDARs (i.e. eGFP-expressing) showed a strong preference for their ipsilateral (unencumbered) forepaw when rearing in a glass cylinder, while those with STN NMDARs knocked out (i.e. cre-eGFP-expressing) recovered the relative use of their affected contralateral forepaw achieving levels similar to vehicle- and eGFP-injected control mice (Figure 6.1.1a; Table 6.1.1). 6-OHDA injection also increased the relative amount of ipsiversive rotations and reduced overall locomotion in the open field when NMDARs were left intact with STN eGFP expression. Cre-eGFP expression in 6-OHDA-injected mice increased their spontaneous locomotion, per distance traveled in the open field test (Figure 6.1.1c-d; Table 6.1.1). Normalization of ipsiversive rotations was achieved in some animals, but not in the population overall (Figure 6.1.1b; Table 6.1.1). Interestingly, though NMDAR knockdown does elevate the strength of cortico-STN transmission in vehicle controls, it did not impact any behavioral metrics relative to animals with intact NMDARs (Figure 6.1.1a-d; Table 6.1.1).
Figure 6.1.1 Knockdown of STN NMDARs ameliorates motor dysfunction in PD mice. (a-d) Contralateral forelimb use (a), ipsiversive rotational behavior (b), and distance traveled in the open field (c, d) were compared in vehicle-injected (dopamine-intact) and 6-OHDA-injected (dopamine-depleted) Grin1lox/lox mice. eGFP-expressing (NMDAR-intact), 6-OHDA-injected mice exhibited relatively impaired contralateral forelimb use (a), elevated ipsiversive rotational behavior (b), and significantly reduced travel in the open field (c, d) compared to eGFP-expressing, vehicle-injected mice. cre-eGFP-expressing (NMDAR-knockdown), 6-OHDA-injected mice exhibited normalized contralateral forelimb use (a) and increased distance traveled in the open field (c, d) relative to eGFP-expressing, 6-OHDA-injected mice. Some 6-OHDA-injected, cre-eGFP-expressing mice also exhibited a normalization of ipsiversive rotational behavior, but the population difference was not significant (b). (a-d) The motor behavior of vehicle-injected mice was not significantly different between eGFP-expressing and cre-eGFP-expressing groups in any metric. (a-c) Population data. (d) Examples of open field activity. *p < 0.05. ns, not significant. Figure components reproduced with permission from Chu et al., 2017.
Table 6.1.1 Knockdown of STN NMDARs ameliorates motor dysfunction in PD mice (relates to Figure 6.1.1).

<table>
<thead>
<tr>
<th>value</th>
<th>treatment group</th>
<th>n (mice)</th>
<th>median [IQ range]</th>
<th>p value (comparison; test stat)</th>
<th>Holm p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Contralateral forepaw use</td>
<td>vehicle + eGFP</td>
<td>11</td>
<td>41.5 [31.4, 48]</td>
<td>( p = 0.52 ) (veh + eGFP vs veh + cre-eGFP; MWU)</td>
<td>( p_h = 0.66 )</td>
</tr>
<tr>
<td></td>
<td>vehicle + cre-eGFP</td>
<td>6</td>
<td>48.6 [36.0, 52.6]</td>
<td>( p = 0.0004 ) (veh + eGFP vs 6-OHDA + eGFP; MWU)</td>
<td>( p_h = 0.0018 )</td>
</tr>
<tr>
<td></td>
<td>6-OHDA + eGFP</td>
<td>7</td>
<td>17.8 [9.8, 22.6]</td>
<td>( p = 0.33 ) (veh + eGFP vs 6-OHDA + cre-eGFP; MWU)</td>
<td>( p_h = 0.66 )</td>
</tr>
<tr>
<td></td>
<td>6-OHDA + cre-eGFP</td>
<td>9</td>
<td>36.4 [28.6, 41.7]</td>
<td>( p = 0.0052 ) (6-OHDA + eGFP vs 6-OHDA + cre-eGFP; MWU)</td>
<td>( p_h = 0.016 )</td>
</tr>
<tr>
<td>% Ipsiversive rotations</td>
<td>vehicle + eGFP</td>
<td>11</td>
<td>42.9 [36.4, 71.7]</td>
<td>( p = 0.94 ) (veh + eGFP vs veh + cre-eGFP; MWU)</td>
<td>( p_h = 0.94 )</td>
</tr>
<tr>
<td></td>
<td>vehicle + cre-eGFP</td>
<td>6</td>
<td>44.2 [32.1, 76.4]</td>
<td>( p &lt; 0.0001 ) (veh + eGFP vs 6-OHDA + eGFP; MWU)</td>
<td>( p_h = 0.0002 )</td>
</tr>
<tr>
<td></td>
<td>6-OHDA + eGFP</td>
<td>10</td>
<td>100 [100, 100]</td>
<td>( p = 0.046 ) (veh + eGFP vs 6-OHDA + cre-eGFP; MWU)</td>
<td>( p_h = 0.098 )</td>
</tr>
<tr>
<td></td>
<td>6-OHDA + cre-eGFP</td>
<td>10</td>
<td>96.7 [61.4, 100]</td>
<td>( p = 0.033 ) (6-OHDA + eGFP vs 6-OHDA + cre-eGFP; MWU)</td>
<td>( p_h = 0.098 )</td>
</tr>
<tr>
<td>Distance traveled (m)</td>
<td>vehicle + eGFP</td>
<td>11</td>
<td>6.4 [4.7, 10.2]</td>
<td>( p = 0.88 ) (veh + eGFP vs veh + cre-eGFP; MWU)</td>
<td>( p_h = 0.88 )</td>
</tr>
<tr>
<td></td>
<td>vehicle + cre-eGFP</td>
<td>6</td>
<td>7.0 [4.4, 9.1]</td>
<td>( p = 0.0015 ) (veh + eGFP vs 6-OHDA + eGFP; MWU)</td>
<td>( p_h = 0.0061 )</td>
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<tr>
<td></td>
<td>6-OHDA + eGFP</td>
<td>10</td>
<td>2.9 [0.9, 4.1]</td>
<td>( p = 0.43 ) (veh + eGFP vs 6-OHDA + cre-eGFP; MWU)</td>
<td>( p_h = 0.85 )</td>
</tr>
<tr>
<td></td>
<td>6-OHDA + cre-eGFP</td>
<td>10</td>
<td>5.9 [3.6, 8.0]</td>
<td>( p = 0.012 ) (6-OHDA + eGFP vs 6-OHDA + cre-eGFP; MWU)</td>
<td>( p_h = 0.035 )</td>
</tr>
</tbody>
</table>
6.2 STN DREADD activation

6.2 Introduction

The rescue of both intrinsic activity and aspects of motor performance with STN NMDAR knockdown provides proof-of-concept for a therapeutic mechanism that restores autonomous firing. However, this strategy is 1) not specific to the firing perturbation (it also preserved cortico-STN connectivity and prevented heterosynaptic plasticity in Chu et al., 2015 and 2017), 2) its therapy has only been shown when knockdown occurs at the time of lesion, and 3) elimination of NMDARs is certain to have unwanted side effects long-term. These and other reasons make STN NMDAR knockdown as a therapeutic intervention in human patients untenable. An alternative and more specific approach was therefore developed using chemogenetics (introduced in Section 4.1).

6.2 Experimental Approach

Animal preparation

Adult male C57BL/6 mice received stereotaxic injections of 1) either 6-OHDA OR vehicle unilaterally in the MFB and 2) AAV-hM3Dq-mCherry into the ipsilateral STN OR immediately dorsal to it in ZI. Two Gabrr3-cre mice were also included in the cylinder test dataset, in which hM3Dq was expressed instead with the cre-dependent virus, AAV-DIO-hM3Dq-mCherry to better restrict hM3Dq expression to the STN due to the paucity of Gabrr3 expression in the surrounding structures. A subset of C57BL/6 animals used to determine the impact of DREADD activation on cortico-STN patterning received injections, in the same hemisphere, of 1) 6-OHDA in the MFB, 2) AAV-hM3Dq-mCherry in the STN, and 3) AAV-ChR2(H134R)-eYFP throughout M1. 2-3 weeks later, animals either underwent behavioral testing OR electrophysiological recording.
**Behavioral assays**

Due to the paired nature of these observations requiring multiple exposures to a behavior paradigm, the cylinder test was used to assess symmetry of forelimb use because it is internally normalized (i.e. scoring does not depend on overall activity, though more accurate measurements are made with more forepaw touches) and the metric is therefore not susceptible to habituation (as is the open field). Mice were tested for akinesia of the forelimb contralateral to the dopamine-depleted hemisphere, and the asymmetry of forelimb use following hM3Dq activation was used to assess its therapy. Animals were briefly anesthetized, weighed, and given an SC injection of either vehicle or CNO (1 mg/kg) and allowed to recover in their home cage. (A small fraction of animals from each group were tested only pre-CNO and post-CNO, receiving no vehicle injection before control trials.) 20-30 minutes after injection, mice were placed in a glass cylinder and their spontaneous rearing behavior was recorded for 5 minutes or until at least 15 paw placements were captured.

**Electrophysiological recordings**

*Ex vivo* recordings were only made from animals that were naïve to CNO (i.e. did not undergo behavioral testing). To determine the impact on autonomous activity, CNO was applied to slices during cell-attached current clamp recording. To test the response of STN neuron short-term plasticity to DREADD activation, post-synaptic currents (PSCs) were evoked by direct electrical stimulation of the internal capsule (IC) during whole-cell voltage clamp recording. IPSCs were measured at a holding voltage of -60 mV in APV and DNQX to block EPSCs. EPSCs were measured at -80 mV in CGP55845 and SR-95531 to block IPSCs. Paired pulses were delivered 100 ms apart and the amplitude of the first pulse, as well as the paired pulse ratio (PPR = PSC2:1), were compared before and after acute CNO (10 µM) application.
6.2 Results

*Chemogenetic activation of STN neurons ex vivo*

To determine the effect of restoring autonomous STN firing on the synaptic patterning of STN neurons and motor dysfunction in PD mice, a chemogenetic rescue strategy was developed (Alexander et al., 2009). Activation of virally expressed hM3Dq with CNO (10 µM) generated a tonic inward current, which increased the frequency and regularity of autonomous STN activity ([Figure 6.2.1a-e; Table 6.2.1](#)). No effect on the amplitude or short-term plasticity of electrically evoked postsynaptic currents in STN neurons was observed ([Figure 6.2.1f, g; Table 6.2.1](#)), confirming that hM3Dq engagement does not acutely alter synaptic input in STN neurons. Depolarization block in STN neurons produced by overstimulation could confer therapy by silencing the structure (Levy et al., 2001; Filali et al., 2004), but *ex vivo* administration of 100 µM CNO (10 X concentration) failed to induce depolarization block in 4 of 4 cells tested ([Table 6.2.1](#)). Together these data demonstrate that the chemogenetic approach we applied specifically restored autonomous firing.
Figure 6.2.1 Chemogenetic activation of STN neurons rescues intrinsic firing. (a) Confocal micrograph depicting expression of hM3Dq-mCherry (red) in NeuN-immunoreactive STN neurons (green, arrowheads). (b, c) Chemogenetic activation of hM3Dq with 10 µM CNO generated a tonic inward current that could be measured under voltage clamp at -60 mV in STN neurons from 6-OHDA- (green) and vehicle- (black) injected mice (b, example from a 6-OHDA-injected mouse slice; c, population). (d, e) Chemogenetic activation of hM3Dq increased the frequency and regularity of autonomous STN activity in ex vivo brain slices from 6-OHDA- and vehicle-injected mice (d, example from a 6-OHDA-injected mouse slice; e, population). (f, g)
Pairs of IPSCs (upper traces; holding voltage, -80 mV) and EPSCs (lower traces; holding voltage, -60 mV) electrically evoked in STN neurons with an interval of 100 ms were unaffected by chemogenetic activation (red) of hM3Dq (f, examples; g, population data). * p < 0.05. n.s., not significant.
Table 6.2.1 Activation of hM3Dq in STN neurons rescues intrinsic firing (relates to Figure 6.2.1).

<table>
<thead>
<tr>
<th>value</th>
<th>treatment group</th>
<th>condition</th>
<th>n (mice/cells)</th>
<th>median [IQ range]</th>
<th>p value (comparison; test stat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-60 mV holding current (pA)</td>
<td>6-OHDA + STN-hM3Dq</td>
<td>control</td>
<td>5/8</td>
<td>-52.2 [-70.2, -33.5]</td>
<td>p = 0.0078 (cntl vs CNO; WSR)</td>
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<tr>
<td></td>
<td></td>
<td>CNO</td>
<td>5/8</td>
<td>-113.2 [-144.6, -65.7]</td>
<td></td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>vehicle + STN- hM3Dq</td>
<td>control</td>
<td>3/6</td>
<td>14.2 [8.9, 18.7]</td>
<td>p = 0.0313 (cntl vs CNO; WSR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CNO</td>
<td>3/6</td>
<td>25.3 [14.8, 34.9]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-OHDA + STN- hM3Dq</td>
<td>control</td>
<td>6/10</td>
<td>1.0 [0, 1.8]</td>
<td>p = 0.0039 (cntl vs CNO; WSR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CNO</td>
<td>6/10</td>
<td>12.6 [4.0, 21.8]</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>vehicle + STN- hM3Dq</td>
<td>control</td>
<td>3/6</td>
<td>0.13 [0.10, 0.19]</td>
<td>p = 0.6875 (cntl vs CNO; WSR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CNO</td>
<td>3/6</td>
<td>0.13 [0.09, 0.15]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-OHDA + STN- hM3Dq</td>
<td>control</td>
<td>5/7</td>
<td>1.1 [0.79, 1.7]</td>
<td>p = 0.016 (cntl vs CNO; WSR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CNO</td>
<td>6/9</td>
<td>0.46 [0.15, 0.70]</td>
<td></td>
</tr>
<tr>
<td>IPSC1 amp (pA)</td>
<td>vehicle or 6-OHDA + hM3D(Gq)-mCherry</td>
<td>control</td>
<td>4/7</td>
<td>0.18 [0.13, 0.38]</td>
<td>p = 0.30 (cntl vs CNO; WSR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CNO</td>
<td>4/7</td>
<td>0.19 [0.15, 0.35]</td>
<td></td>
</tr>
<tr>
<td>IPSC PPR</td>
<td>vehicle or 6-OHDA + hM3D(Gq)-mCherry</td>
<td>control</td>
<td>4/7</td>
<td>0.83 [0.77, 0.89]</td>
<td>p = 0.16 (cntl vs CNO; WSR)</td>
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<tr>
<td></td>
<td></td>
<td>CNO</td>
<td>4/7</td>
<td>0.85 [0.82, 0.97]</td>
<td></td>
</tr>
<tr>
<td>EPSC1 amp (pA)</td>
<td>vehicle or 6-OHDA + hM3D(Gq)-mCherry</td>
<td>control</td>
<td>4/7</td>
<td>-0.18 [-0.37, -0.11]</td>
<td>p = 0.078 (cntl vs CNO; WSR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CNO</td>
<td>4/7</td>
<td>-0.16 [-0.32, -0.11]</td>
<td></td>
</tr>
<tr>
<td>EPSC PPR</td>
<td>vehicle or 6-OHDA + hM3D(Gq)-mCherry</td>
<td>control</td>
<td>4/7</td>
<td>0.86 [0.76, 1.04]</td>
<td>p = 1 (cntl vs CNO; WSR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CNO</td>
<td>4/7</td>
<td>0.74 [0.73, 1.0]</td>
<td></td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>6-OHDA + STN- hM3Dq</td>
<td>100 µM CNO</td>
<td>2/4</td>
<td>30 [22.3, 35.7]</td>
<td>(all values nonzero)</td>
</tr>
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</table>
Abnormally persistent and coherent beta band (13-30 Hz) activity in the cortex and STN has been consistently linked to the motor symptoms of PD and its experimental models (Gatev et al., 2006; Gale et al., 2008; Kühn et al., 2008; Bronte-Stewart et al., 2009; Tachibana et al., 2011; Shimamoto et al., 2013; Delaville et al., 2015). Given the decorrelating impact of intrinsic activity on synaptic input (Wilson 2013), we hypothesized that chemogenetic restoration of autonomous STN activity would diminish the capability of beta frequency optogenetic M1 stimulation to pattern spiking in STN neurons in brain slices from PD mice. Indeed, the number of “synaptically-driven” APs during M1-STN stimulation (defined as the APs/10 ms bin during stimulation that exceeded the mean + 3SD APs/10 ms bin during the control period) was universally reduced following hM3Dq activation and restoration of intrinsic firing (Figure 6.2.2; Table 6.2.2). This result implies that the rescue of autonomous STN activity in PD mice reduces the impact of M1-STN input as a proportion of STN output and may therefore oppose pathological synchronous oscillations.
Figure 6.2.2 Chemogenetic activation of STN neurons reduces synaptic patterning by cortical inputs. (a-d) The patterning of STN activity by motor cortical inputs optogenetically stimulated at 20 Hz for 1 second (blue) in ex vivo brain slices from 6-OHDA-injected mice was reduced by chemogenetic activation of hM3Dq in STN neurons (a, example cell-attached recordings; b, population peristimulus time histogram (magenta line, mean; cyan line, mean + 3SD); c, d, population data). * p < 0.05. n.s., not significant.
Table 6.2.2 Chemogenetic activation of STN neurons reduces synaptic patterning by cortical inputs (relates to Figure 6.2.2).

<table>
<thead>
<tr>
<th>value</th>
<th>treatment group</th>
<th>condition</th>
<th>n (mice/cells)</th>
<th>median [IQ range]</th>
<th>p value (comparison; test stat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synaptic drive (spikes)</td>
<td>6-OHDA</td>
<td>control</td>
<td>7/10</td>
<td>6.0 [4.0, 14.6]</td>
<td>p = 0.0020 (cntl vs CNO; WSR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CNO</td>
<td>7/10</td>
<td>0.03 [0, 1.5]</td>
<td></td>
</tr>
<tr>
<td>% of spikes driven</td>
<td>6-OHDA</td>
<td>control</td>
<td>7/10</td>
<td>10.8 [4.4, 50.4]</td>
<td>p = 0.0020 (cntl vs CNO; WSR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CNO</td>
<td>7/10</td>
<td>0.1 [0, 1.2]</td>
<td></td>
</tr>
</tbody>
</table>
It is well established that the manifestation of PD symptoms often correlates with abnormally persistent and synchronous oscillations in the STN, and the alleviation of deficits can be achieved by disrupting those oscillations (Gatev et al., 2006; Gale et al., 2008; Kühn et al., 2008; Bronte-Stewart et al., 2009; Joundi et al., 2012; Benabdallah et al., 2009; McConnell et al. 2012; Zaidel et al., 2010; Eusebio et al., 2012; Whitmer et al., 2012; Agnesi et al., 2013; Shimamoto et al., 2013). In light of our finding that chemogenetic restoration of intrinsic firing reduced cortico-STN patterning, we predicted that *in vivo* activation of hM3Dq in PD mice would ameliorate motor deficits.

To first demonstrate that CNO administration *in vivo* does indeed elevate activity in STN neurons and target structures, the depolarization-induced expression of c-fos was immunohistochemically detected in mice that received either vehicle or CNO 90 minutes before their brain tissue was harvested and sliced (Morgan et al., 1987). SN-containing slices were also reacted against antibodies to parvalbumin (PV, a Ca\(^{2+}\)-binding protein) to identify likely recipient neurons of STN inputs: the GABAergic output neurons of the basal ganglia. As predicted, mice in which STN neurons were chemogenetically activated with CNO *in vivo* showed a higher density of hM3Dq-mCherry-expressing, c-fos+ neurons in the STN and PV-expressing, c-fos+ neurons in the SN than those that received vehicle injections (**Figure 6.2.3; Table 6.2.3**), demonstrating that SC CNO administration at 1 mg/kg elevates STN neuron activity and transmission *in vivo.*
Figure 6.2.3 Chemogenetic activation of STN neurons in vivo increases immediate early gene expression in STN neurons and their targets in the SN. (a-c) The density of hM3Dq-mCherry-expressing STN neurons that expressed c-fos was elevated in dopamine-intact and -depleted mice that were treated with CNO versus those treated with vehicle (a, b, examples; c, population data). (d-f) The density of PV-immunoreactive SN neurons that expressed c-fos was elevated in dopamine-intact and -depleted mice that were treated with CNO versus those treated with vehicle (d, e, examples; f, population data). * p < 0.05.
**Table 6.2.3** Chemogenetic activation of STN neurons *in vivo* increases immediate early gene expression in STN neurons and their targets in the SN (relates to Figure 6.2.3).

<table>
<thead>
<tr>
<th>value</th>
<th>treatment group</th>
<th>condition</th>
<th>n (mice/sites)</th>
<th>median [IQ range]</th>
<th>p value (comparison; test stat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>density of cFos+ neurons</td>
<td>vehicle +</td>
<td>vehicle</td>
<td>3/18</td>
<td>0 [0, 8.9]</td>
<td>p &lt; 0.0001 (vehicle vs CNO; MWU)</td>
</tr>
<tr>
<td>in STN (10^3.mm^-3)</td>
<td>hM3D(Gq)</td>
<td>CNO</td>
<td>3/18</td>
<td>100.4 [43.5, 144.1]</td>
<td></td>
</tr>
<tr>
<td>density of PV+ neurons</td>
<td>6-OHDA +</td>
<td>vehicle</td>
<td>3/18</td>
<td>0 [0, 0]</td>
<td>p &lt; 0.0001 (vehicle vs CNO; MWU)</td>
</tr>
<tr>
<td>in SNr (10^3.mm^-3)</td>
<td>hM3D(Gq)</td>
<td>CNO</td>
<td>3/18</td>
<td>129.5 [79.81, 180.6]</td>
<td></td>
</tr>
<tr>
<td>density of cFos+ neurons</td>
<td>vehicle +</td>
<td>vehicle</td>
<td>3/18</td>
<td>0 [0, 0]</td>
<td>p &lt; 0.0001 (vehicle vs CNO; MWU)</td>
</tr>
<tr>
<td>in SNr (10^3.mm^-3)</td>
<td>hM3D(Gq)</td>
<td>CNO</td>
<td>3/18</td>
<td>37.1 [0, 44.8]</td>
<td></td>
</tr>
<tr>
<td>density of PV+ neurons</td>
<td>6-OHDA +</td>
<td>vehicle</td>
<td>3/18</td>
<td>0 [0, 33.4]</td>
<td>p &lt; 0.0001 (vehicle vs CNO; MWU)</td>
</tr>
<tr>
<td>in SNr (10^3.mm^-3)</td>
<td>hM3D(Gq)</td>
<td>CNO</td>
<td>3/18</td>
<td>86.3 [44.1, 105.4]</td>
<td></td>
</tr>
</tbody>
</table>
To test the therapeutic effectiveness of chemogenetically activating STN neurons in PD mice, the cylinder test for forelimb use asymmetry was administered following vehicle, CNO, or no injection. In unilateral MFB vehicle-injected, dopamine-intact mice, forelimb usage was symmetric and chemogenetic activation in mice that expressed hM3Dq-mCherry in the STN had no consistent effect (Figure 6.2.4a, b; Table 6.2.4). Mice that received 6-OHDA injections (and exhibited >80% loss of ipsilateral striatal TH immunoreactivity) consistently used the forepaw ipsilateral to the toxin injection preferentially during control conditions, and used their contralateral forepaw in significantly lower proportions compared with MFB vehicle-injected controls (Figure 6.2.4a; Table 6.2.4). Within 30 minutes of SC administration of CNO (1 mg/kg), unilateral 6-OHDA-injected mice with ipsilateral STN hM3Dq-mCherry expression increased their relative usage of the contralateral Parkinsonian forelimb and decreased their relative usage of the ipsilateral forelimb compared to baseline activity, while MFB sham-injected controls showed no consistent change in forepaw use proportion following chemogenetic activation of the STN (Figure 6.2.4a-c; Table 6.2.4; Video S1). In unilateral MFB 6-OHDA-injected, dopamine-depleted mice with hM3Dq-mCherry expressed ipsilaterally in regions adjacent to but not including the STN, baseline forelimb usage was also profoundly asymmetric, and the proportion of contralateral forelimb use increased following CNO administration (Figure 6.2.4d; Table 6.2.4). However, chemogenetic activation of the STN had a significantly greater impact on forelimb usage (measured as the absolute change from baseline in the proportion of contralateral forelimb use) when compared with PD mice in which only STN-adjacent structures were chemogenetically activated (Figure 6.2.4e; Table 6.2.4). Together these data suggest that chemogenetic activation of the STN in unilateral 6-OHDA-injected mice rescues usage of the contralateral Parkinsonian forelimb during vertical exploratory behavior, indicating that restoration of autonomous STN activity can ameliorate Parkinsonian motor dysfunction. These results further bolster our hypothesis that autonomous STN activity disruption following
dopamine loss promotes synchronous circuit oscillations and correlated PD symptoms, because chemogenetically rescuing intrinsic firing reduces the synaptic drive of the STN and ameliorates motor deficits.
Figure 6.2.4 Chemogenetic activation of STN neurons in vivo ameliorates motor deficits in PD mice. (a) In the cylinder test, hemiparkinsonian mice exhibited asymmetric forelimb use favoring the forepaw ipsilateral to the dopamine-depleted hemisphere, using their contralateral forepaw a significantly smaller proportion of the time during baseline measurements when compared with MFB vehicle-injected controls. (b) MFB vehicle-injected mice showed no consistent change in forelimb usage following chemogenetic activation of hM3Dq-mCherry expressed in the STN and zona incerta (ZI) with CNO (SC 1 mg/kg; left, hM3Dq-mCherry expression (red); right, population data, micrograph example(s) highlighted). (c, d) Proportional use of the contralateral forepaw was significantly increased by chemogenetic activation of the STN (c) or ZI alone (d; panels c and d as for b). Viral expression was restricted to the STN using cre-dependent DIO-hM3Dq-mCherry expressed in 2 Gabrr3-cre mice.
that received MFB 6-OHDA injections (e.g. example 1 in c). (e) The normalization of forepaw use in 6-OHDA-treated mice was significantly greater when chemogenetic activation involved the STN compared with mice in which solely STN-adjacent structures were involved (as measured by the absolute change in % contralateral forepaw use during baseline and following CNO injection).

* p < 0.05. n.s., not significant.
Table 6.2.4 Chemogenetic activation of STN neurons \textit{in vivo} ameliorates motor deficits in mice (refers to Figure 6.2.4).

<table>
<thead>
<tr>
<th>value</th>
<th>treatment group</th>
<th>condition</th>
<th>n (mice)</th>
<th>median [IQ range]</th>
<th>( p ) value (comparison; test stat)</th>
<th>Holm ( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Contralateral forepaw use</td>
<td>6-OHDA + STN- hM3Dq</td>
<td>CNO - cntl</td>
<td>13</td>
<td>15.7 [7.8, 21.6]</td>
<td>( p &lt; 0.030 ) (6-OHDA + STN-hM3Dq vs 6-OHDA + ZI-hM3Dq, MWU)</td>
<td>( p_h = 0.030 )</td>
</tr>
<tr>
<td>% Contralateral forepaw use</td>
<td>6-OHDA + ZI- hM3Dq</td>
<td>CNO - cntl</td>
<td>9</td>
<td>6.7 [1.7, 11.9]</td>
<td>( p = 0.0012 ) (vehicle + STN-hM3Dq vs 6-OHDA + STN-hM3Dq, MWU)</td>
<td>( p_h = 0.0023 )</td>
</tr>
</tbody>
</table>

\( \text{Contralateral forepaw use} \)
6.3 Conclusions

While contrary to Albin et al.’s 1989 predictions that elevating STN firing in the PD brain would have a deleterious effect on symptoms, these results implying the therapeutic benefit of artificially restoring intrinsic STN firing chemogenetically (or preventing its loss, e.g. with STN NMDAR knockdown) support the field’s shifting consensus that the pattern dynamics of circuit activity in the basal ganglia are more important for executing their functions than overall rates of neuronal firing (introduced in Section 1.1 and discussed further in Section 7.2). For indeed, both the stimulation (Benabid et al., 2009; Wichmann and Delong, 2006) and silencing (Levy et al., 2001; Yoon et al., 2014) or ablation (Bergman et al., 1990) of the STN show therapy, all of which achieve the outcome of reducing the propagation of synchronous circuit oscillations through the STN. Recent work by Bhattacharya et al. (2018) has also produced results confirming the therapeutic effects of blocking NMDAR activity in the STN of MPTP monkeys, suggesting that preventing NMDAR-dependent plasticity provides an additional means of targeting the STN in PD. However, this GluN2D-specific effect may be relatively unreliable, as GluN2D-containing NMDAR expression was also reduced in dopamine-depleted rodents (Bhattacharya et al., 2018).

Though we had access to a limited number of transgenic Gabrr3-cre mice in which cre expression is fairly restricted to the STN in the vicinity of the injection, the bulk of the behavioral experiments were performed necessarily in C57BL/6 mice. Therefore, expressing hM3Dq-mCherry in the STN exclusively was not possible (Section 6.2). Contamination of immediately adjacent areas included the ZI just ventral to the thalamus and the parasubthalamic nucleus (PSTN) medial to the STN—both of which have been shown to participate in motor function.
First described by Auguste Forel as “a region of which nothing certain can be said” (Mitrofanis, 2005), recent attention to the ZI has been generated for its myriad functions in 1) promoting sleep with activation of Lhx6-positive GABAergic neurons of the ventral (v) ZI (Liu et al., 2017); 2) promoting cortical neuron development via a GABAergic projection to layer 1 in mice (Chen and Kriegstein, 2015); and 3) producing binge-like eating with stimulation of GABAergic ZI projections to the paraventricular thalamus (Zhang and van den Pol, 2017). But among its vast functions and brain region associations are a number of connections related to locomotion and posture, as indicated by Edwards and Isaacs’s 1991 lesion study in rats (Edwards and Isaacs, 1991). Indeed, anatomical and functional evidence suggest the ZI participates in the dopamine-modulated cortico-basal ganglia-thalamo-cortical circuit, and makes (often reciprocal) connections in the PPN, midbrain, cerebellum, and spinal cord, the majority of which involve the caudal (c) ZI (reviewed by Mitrofanis, 2005). According to Heise and Mitrofanis (2004), the ZI’s efferents to the PPN, SN, and GPe are mostly glutamatergic with sparse GABAergic projections. Most BG cells projecting to the ZI (per retrograde tracer injections in rats) originate in SNc, SNr, with smaller fractions in GPi and GPe; there were none in striatum or STN (Heise and Mitrofanis 2004). ZI neurons also exhibit a sensitivity to dopamine loss: 1) there are TH+ cells and TH+ terminals (from SNc) in ZI (Heise and Mitrofanis, 2004), 2) there is a reduction in PV expression among ZI neurons from the lesioned hemisphere in 6-OHDA-treated rats (Heise and Mitrofanis, 2005), and 3) ZI neurons in rats show elevated activity following dopamine depletion (Pérrier et al., 2000). Furthermore, studies of DBS electrode placement in PD patients have revealed that there is significant therapeutic benefit to stimulating in ZIP (Henderson et al., 2002; Voges et al., 2002; Plaha et al., 2006; Khan et al., 2011; Blomstedt et al., 2018). Thus, there is ample evidence to suggest chemogenetic stimulation of the ZI could influence motor output in PD mice—putatively through its glutamatergic projections to the SN, PPN and GPe (Heise and Mitrofanis, 2004). However, the ZI’s proposed global function as a “limbic-motor integrating center”
(providing short-circuited feedback between internal and external cues linking arousal, visceral responses, attention, and locomotion; Mitrofanis 2005) can also engage unintended consequences when electrically stimulated (e.g. sleepiness or binge eating; Liu et al., 2017; Zhang and van der Pol, 2017). The identities and properties of ZI neurons would be a productive area of further study that could help identify additional therapeutic targets for PD. Yet the present study suggests the restoration of intrinsic STN activity with chemogenetic activation is more effective than chemogenetic activation of ZI alone (Figure 6.2.4).

The PSTN comprises a little-studied collection of neurons extending from the lateral hypothalamic area lying just medial to the caudal half of the STN that express β-preprotachykinin (β-PPT) mRNA, which encodes substance P (Wang and Zhang, 1995). Extensive mapping studies indicate the PSTN transmits information from forebrain regions to modulate hindbrain autonomic functions, particularly those related to gustatory and visceroceptive inputs (reviewed in detail by Goto and Swanson, 2004; Kita et al., 2014). Additionally, PSTN neurons have been found to regulate cardiovascular function, as stimulation of these substance P-expressing cells reduces blood pressure and heartrate (Allen and Cechetto, 1992, 1993). Interestingly, the dorsomedial edge of the STN that abuts the PSTN (the medial cap, STNmc) has been proposed to influence motor control through its projections to the lateral striatal fundus, rostral substantia innominata, ventral GP, and the caudal extent of the dorsolateral SN (Goto and Swanson, 2004). Thus, in addition to the classically-motor lateral portion of the STN, this area in the medial subthalamic region may also contribute to locomotion, and its chemogenetic stimulation may influence motor performance in PD mice. A more restricted and cell-specific approach—e.g. using retro-AAV expressing cre to target STN neurons projecting to GPe/GPi/SNr with injection of a cre-dependent DREADD—would help elucidate the specific contributions of each subthalamic region to therapy.
CHAPTER 7: DISCUSSION & FUTURE DIRECTIONS

The use of mouse models to study neurological disease has enabled investigation into normal and pathological processes in the brain with a revolutionary degree of control and detail. With a goal of elucidating disease mechanisms to discover targets and treatments to benefit humans, a critical consideration of their functions and caveats is imperative for the effective translation to medicine. Herein I discuss theories and further questions surrounding the tenets laid out by this thesis: 1) D2-SPN hyperactivity in the absence of dopamine leads to disinhibition of STN neurons, making them more susceptible to NMDAR activation by cortical inputs of the hyperdirect pathway that triggers autonomous activity disruption; 2) mitochondrial oxidation and K$_{ATP}$ channel activation underlie the downregulation of STN activity in PD mice; and 3) restoration of intrinsic firing with chemogenetic activation of STN neurons reduces the impact of cortico-STN inputs and ameliorates Parkinsonian motor symptoms.

7.1 Maladaptive plasticity

The circuit-dependent triggers of autonomous STN activity disruption—i.e. elevated D2-SPN activity disinhibiting the STN, thereby facilitating NMDAR-dependent K$_{ATP}$ channel activation—hint that the hyperpolarizing response of STN neurons may be an adaptation to its augmented excitation following loss of dopamine. In a healthy brain, the GPe provides a fairly tonic level of inhibition to the STN (Mallet et al., 2008b; Galvan and Wichmann, 2008) and mediates the impact of cortical excitation through hyperpolarization and shunting inhibition (Fujimoto and
Kita, 1993; Maurice et al., 1999; Bevan et al., 2002; Baufreton et al., 2005; Baufreton et al., 2009; Atherton et al., 2010). When DA is scarce and D2-SPNs are consequently more excitable, GPe activity becomes periodic and phase-locked with cortical oscillations such that cortico-STN excitation coincides with periods of STN disinhibition, amplifying the impact of glutamatergic transmission (Magill et al., 2001; Mallet et al., 2008b). Excessive Ca²⁺ influx through NMDARs can impair cell metabolism through mitochondrial oxidative damage and ultimately induce apoptotic and necrotic cell death (Green and Reed, 1998; Pivavarova and Andrews, 2010). It is therefore unsurprising that, in response to elevated NMDAR activation, signaling pathways that activate K_ATP channels are engaged and slow the STN’s energetically costly autonomous activity. This function is further supported by Parsons and Hirasawa’s (2010) work in orexin neurons, in which they found K_ATP channel activation during glucose deprivation has a neuroprotective effect. With K_ATP channel blockade during glucose deprivation, the AHP magnitude decreased and caused an eventual loss of firing. While K_ATP channel activation may be an adequate adaptation to resist glutamate excitotoxicity in the PD brain (e.g. Carvalho and Nikkhah (2001) report no loss of STN neurons following 6-OHDA treatment in rats), the therapeutic benefit of chemogenetic rescue argues that the apparent homeostatic reduction of intrinsic firing following dopamine loss is actually *maladaptive* in the context of the Parkinsonian circuit.

### 7.2 Firing rates and patterns

There is mounting evidence in animal research suggesting that the pattern of activity and correlation with other brain areas, rather than overall firing rates alone (per Albin et al., 1989) encode movement initiation and choice in the cortico-basal ganglia-thalamo-cortical circuit (e.g. Bevan et al., 2002; Mallet et al., 2008b; Engle and Fries 2010; Muthukumaraswamy 2010; Rosin et al., 2011; reviewed in Wichmann and DeLong, 1996). Indeed, while the classical rate model
would predict elevation of STN activity to further impair movement by exciting inhibitory basal
ganglia output nuclei, our data show that chemogenetic activation of the STN instead ameliorates
deficits, putatively by altering its patterning by/correlation with the cortex. This result is
consistent with reports that reductions in STN synchronization (e.g. during DBS and/or L-DOPA
treatment) coincide with symptom improvement (Gatev et al., 2006; Hammond et al., 2007;
Kühn et al., 2009; López-Azcárate et al., 2010). The mechanisms by which DBS decorrelates STN
activity and improves symptoms are unclear; reports implicate direct STN/GPi neuronal
excitation (Toleikis et al., 2012) or inhibition (Filali et al., 2004), antidromic stimulation (Kang
and Lowery, 2014; Li et al., 2007, 2012), and the stimulation of astroglia (Tawfik et al., 2010;
Vedam-Mai et al., 2012) or of nearby brain structures/fibers (Breit et al., 2004; Gradinaru et al.,
2009; reviewed in Chiken and Nambu, 2016).

This study presents yet another potential mechanism by which DBS promotes normalized circuit
activity—by providing a source of AP generation extrinsic to (pathologically patterned) synaptic
inputs that resists entrainment by them. More specifically, chemogenetic restoration of intrinsic
STN firing makes the neurons’ output (per their AP generation, Figure 6.2.2) no longer
dominated by abnormally synchronous excitatory input, but instead provides a mosaic of relays
that integrate glutamatergic and GABAergic signals over an independently-generated baseline
firing rate and pattern. A tonic level of autonomous activity enables bidirectional modulation of
the neuron’s excitability and the temporal encoding of incoming signals, either advancing or
delaying (or sometimes negating) synaptically generated APs depending on the neuron’s activity
immediately prior (Wilson 2013). In this way, the autonomous activity of STN neurons also
introduces a kind of filter that meets incoming cortical oscillations with a gauntlet of its own
oscillatory patterns. The integration of synchronous hyperdirect pathway input with a
heterogeneous population of intrinsic oscillators would be predicted to “blur” the incoming
signals. One could imagine that this type of integration would reduce the impact of coincidental co-activations from global background oscillations and sharpen or strengthen any intentional signals that persist through the STN’s processing. On the contrary, an overly coherent signal transmitted to a glutamatergic nucleus of quiescent neurons with an entire complement of Na\(^+\) channels available will respond readily to excitatory input, locking step to it and transmitting the signal, relatively unaltered, to its targets. However, there can be virtually no information in such a coherent and unchanging signal. The results in Section 6.1 suggest that the chemogenetic restoration of intrinsic STN activity does indeed diminish “synaptic drive” of the STN, largely by reducing the relative contribution of cortical input to the STN’s firing activity and output. Thus, despite predictions by the classical model that elevating firing rates in the STN would worsen motor deficits (Albin et al., 1989), these data argue the importance of temporal patterning (per the relationship between cortical input and STN output) of STN neurons in motor circuit function.

### 7.3 PD model refinement

The advantages of using a mouse model for studying cellular- and circuit-level disease processes have been touted throughout this thesis. Their short gestation (20 days) and maturation times (45 days), combined with the plethora of transgenic lines continuously being produced makes mice an extremely fruitful tool. However, while certain phenotypes can be reproduced—like akinesia (per reduced voluntary movement on the impaired side in hemiparkinsonism; e.g. Schwarting et al., 1991; Dowd et al., 2005; reviewed by Schwarting and Huston, 1996), elevated synchronous oscillations (reviewed in Quiroga-Varela et al., 2013), and susceptibility to LID (Lundblad et al., 2004)—the acute degeneration of dopaminergic cells using a neurotoxin does not faithfully track the course and symptomology of the human disease.
A model of progressive dopamine loss like the MitoPark strain (introduced in Section 1.2) is likely to 1) better replicate the physiology of the human disease, 2) further inform the evaluation of other PD models to determine the caveats of each regarding their relevance to idiopathic PD, and 3) allow resolution on a finer timescale for the development of parkinsonian pathologies and the potential for pre-symptomatic treatment. As demonstrated by Chu et al., (2015, 2017) and this thesis, there are numerous changes to the synaptic and cell-autonomous physiology in the STN of 6-OHDA-treated mice: GPe-STN synapses are heterosynaptically potentiated (via cortical activation of NMDARs), cortico-STN inputs are reduced in number, and intrinsic STN firing is disrupted. But when and to what degree these plasticities are engaged relative to and due to one another is not clear, but important for thoroughly understanding the causes of parkinsonian deficits stemming from abnormal STN activity. Of course, a model in which Tfam knockdown in dopamine neurons is inducible would eliminate questions of the knockdown’s effects during development and enable the adult onset of the mitochondrial dysfunction (e.g. with doxycycline-dependent lentiviral expression, which is both inducible and reversible; Matsushita et al., 2013).

Animals that undergo progressive dopamine depletion have also been developed to an extent in toxin models. Sauer and Oertel (1996) achieved this by injecting 6-OHDA into the striatum in rats, and observed a progressive loss of SNc dopamine neurons over 16 weeks. The Gittis lab is currently pursuing a progressive PD model in which 6-OHDA is titrated via canulae into the MFB at low concentrations (5 injections of 0.75 µg) over a period of weeks to months to mimic the pattern of DA neuron degeneration in idiopathic PD (discussed in Section 1.2). This model allowed them to distinguish a progressive (and, at 70% striatal DA loss, acute) deterioration of some behaviors (e.g. cylinder test and spontaneous locomotion in the open field), while motor coordination (assessed in the vertical pole task) was less affected overall than in acutely lesioned models (Willard et al., 2015). Thus, though more technically involved and therefore variable than
the MitoPark model, the gradual 6-OHDA toxin model also holds promise for dissecting the progression of circuit abnormalities and symptoms secondary to the eventual loss of DA neurons, and on an experimenter-defined timescale that can be custom designed to suit the question being addressed.

7.4 “Chemo-DBS”

Taken together, our findings argue that restoring autonomous firing to STN neurons reduces both synaptic patterning of STN activity and akinetic Bradykinetic symptoms in PD models. In contrast to current therapeutic strategies, which either lesion the STN or non-specifically (and synchronously) stimulate the STN and its afferent and efferent structures, the chemogenetic approach described here specifically corrects a deficit in STN neuron physiology that is caused by the loss of dopamine in an attempt to normalize circuit activity and motor function. While at odds with the classical view that elevated STN activity underlies the motor symptoms of PD (Albin et al., 1989), our findings align with the emerging view that symptoms stem from aberrant synchrony and patterning and that targeting this problem may be a more effective therapeutic strategy for PD patients (Hammond et al., 2007; Galvan et al., 2015).

Of course, this type of virally-delivered experimental tool needs considerable refinement before even proposing its use in humans. A more comprehensive understanding needs to be achieved first in animal models of 1) viral vector-host interactions, 2) the ligand effector’s safety and efficacy, 3) the actuator protein’s mechanisms of action, and 4) the longevity of its potential therapy in the context of a parkinsonian circuit. Indeed, researchers are actively working on safe and effective viral serotypes for gene therapy treatments—a task fraught with challenges like natural immunity in the human population, BBB permeability, faithful targeting, and appropriate
dosing; reviewed in Lykken et al., 2018). A more specific and unique protein-ligand interaction specialized for elevating intrinsic STN activity would be desirable as well to moderate off-target effects on endogenous cellular mechanisms (like the downstream signaling of GPCRs). An ion channel or pump, for example, may have broader applications and fewer corollary effects (with the obvious exception of Ca\textsuperscript{2+} influx, which is a second messenger in itself and is one of the hypothesized catalysts of autonomous activity disruption to begin with, per Section 4.2). STN-specific expression in patients will be difficult to achieve without the transgenic tools we have in mice, but a real-time intraoperative MRI guidance system is being developed in nonhuman primates to improve targeting (e.g. by Vermilyea et al., 2017 to inject induced pluripotent stem cell-derived DA progenitor cells into rhesus monkeys as a PD treatment). Restricting off-target effects of the chemogenetic ligand is also important—a challenge already being addressed in the DREADD family of receptors, as emerging studies of the “designer drug” CNO suggest that it is not as exclusive as previously presumed. CNO is converted to clozapine (and norclozapine) in the mammalian brain, and new autoradiographical and behavioral evidence points to clozapine as a potent actuator of DREADD engagement in vivo (Gomez et al., 2017). This caveat of CNO brain pharmacokinetics raises concerns of clozapine’s binding to endogenous receptors, and requires the effects of CNO in non-DREADD-expressing animals be assessed alongside experimental groups to distinguish the off-target effects of clozapine from the targeted effects of chemogenetic activation. Careful selection of an appropriate CNO dose and controls for non-DREADD effects were therefore paramount to demonstrating the specific impact of chemogenetic activation in STN neurons in this study, hence the inclusion of non-DREADD-expressing mice in paradigms involving in vivo CNO administration (e.g. for experiments in Figures 4.1.2 and 6.2.2).

Ex vivo uses of DREADDs commonly cite 1-10 µM CNO for bath application in a brain slice preparation, and this concentration produces potent and consistent increases in STN firing rates
in our hands. From the pharmacokinetics of CNO measured in humans, Rei et al (2015) calculated that a 2.5 mg/kg injection in a ~30-g mouse would yield a peak brain concentration of CNO around 12 µM, suggesting in vivo injections at this order of magnitude are expected to produce comparable levels to its ex vivo application. In animals lacking DREADD expression, 1 mg/kg CNO (used in this study) has not been observed to evoke changes in spontaneous locomotion in rodents during the first hour following injection (the time window for motor assessment in this study), but does produce observable behavioral changes in DREADD-expressing mice. For example, Gomez et al., (2017) demonstrated that chemogenetic inhibition with hM4Di in D1-expressing SPNs with 1 mg/kg CNO caused a significant reduction in locomotor activity during the first hour of open field exploration (compared with vehicle-injected controls), while the same dose of CNO in mice lacking DREADD expression did not affect locomotion during the first hour, suggesting the reduction observed in hM4Di-expressing mice was due to engagement of the receptor. However, a less dramatic yet significant reduction in spontaneous activity during the second hour of exposure was observed in WT mice following 1 mg/kg CNO (though MacLaren et al (2016) found no such effect on open field activity in WT rats during the 2 hours following a 1 or 2 mg/kg CNO dose). The mouse behavioral data presented by Gomez et al. indicate there is a rapid activation of DREADDs by CNO in the first hour, followed by additional non-DREADD effects that reduce locomotion in the second hour. It remains unclear what action CNO specifically is having on CNS-expressed DREADDs in vivo, as low doses (0.01 mg/kg) of clozapine elicited a similar (though less robust) reduction in distance traveled during the first hour after injection in D1-SPN hM4Di-expressing mice (with no corresponding effect in WT controls). However, whether the effective DREADD ligand in vivo is CNO or clozapine, it appears the potential off-target (i.e. non-DREADD) impacts on mouse activity emerge only 2-3 hours following its injection. Indeed, Raper et al.’s (2017) observations in monkeys show a rapid (within 30 minutes) elevation of CSF CNO concentration following 3 and 10 mg/kg doses, and delayed elevations in
CSF clozapine concentrations that peak at 2-3 hours post-injection, which leaves open the possibility that either CNO or very low concentrations of clozapine are responsible for the chemogenetic effect in the first hour, while subsequent time points may be dominated by an abundance of clozapine that then engages lower-affinity receptors. Considered together with our control data, these studies support the supposition that motor responses elicited by chemogenetic manipulation of the basal ganglia within the first hour of a 1 mg/kg CNO injection are due to activation of the DREADDs and not off-target effects of CNO or its metabolites.

Thus, while the chemogenetic tools used herein are not yet appropriate for use in humans, and further understanding of this approach’s normalizing effects on the parkinsonian circuit are needed, this study provides a proof of concept for a novel theory and therapeutic approach to treating the symptoms of advanced PD by restoring intrinsic firing to neurons of the STN.

### 7.5 The new and the improving

The results of this thesis implore a plethora of questions and future avenues of investigation that exceed the purview (and sanity) of a PhD student. But for posterity’s sake, here are a few potential topics of further study:

1. the relative time-course of STN plasticities and intracellular ROS signaling during the development of parkinsonism (better studied in a progressive model);
2. the specific subunits that contribute to the NMDAR-dependent plasticities of the STN in PD and their distribution within the STN neuron, which could help more specifically identify the circuit behavior that engages the mechanism (e.g. if receptors are synaptic vs exgrasynaptic) and potentially target them for therapy;
3. whether STN K$_{ATP}$ channel knockdown in PD mice results in cell death, which would speak to the adaptive nature of this mechanism;

4. the G$_q$-mediated signaling in STN neurons that capitulates the elevation/restoration of intrinsic oscillations, which could reveal potential pharmacological interventions (though less specific, may be more readily determined safe for clinical administration);

5. the long-term effects of chronic chemogenetic activation of the STN in PD models: expression maintenance, cell health, adaptative plasticity, and behavioral outcomes.

Improvements to the methodological approaches used in this body of work could open additional avenues of inquiry to exploration. Most notably, a better PD model, more specific expression and directed activation of a chemogenetic compound, and more sophisticated and varied behavioral observations would greatly augment our assessment of the therapeutic potential of restoring intrinsic firing to STN neurons to ameliorate parkinsonian symptoms. The continued development of advanced optical detection tools (from voltage-sensitive dyes to fluorescent indicators of ATP and ROS), genetic tools (from cre lines and disease models to viral vectors), and molecular compounds (from designer receptors to activity-dependent drugs) will also help answer questions to bolster or refute current theories. The future of neuroscience is exciting, but perhaps more importantly, the future’s PhD students are going to cure PD by the time I get it.
References


ventral hippocampus to the lateral septum and medial prefrontal cortex. *Neuropsychopharm* 42(8):1715–1728.


Rueda, C.B., Llorente-Folch, I., Traba, J., Amigo, I., Gonzalez-Sanchez, P., Contreras, L., ... and Satrustegui, J. (2016) Glutamate excitotoxicity and \(\text{Ca}^{2+}\)-regulation of respiration: Role of the \(\text{Ca}^{2+}\)-activated mitochondrial transporters (CaMCs). *Biochimica et Biophysica Acta - Bioenergetics* 1857(8):1158–1166.


