12-2017

Pitx3Null Mutant (Striatal Dopamine-Deficient) Mice Have Exaggerated Spiny Projection Neuron Responses to l-DOPA and D1 Agonism and Lack Baseline Striatonigral Spiking

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Abstract

L-3,4 dihydroxyphenylalanine (l-DOPA) strongly stimulates motor activity in parkinsonian patients and animal models of Parkinson's disease. Severe striatal dopamine (DA) loss characterizes Parkinson's disease and its animal models. Given the canonical rate model of Parkinson's Disease pathophysiology based on differences in DA pharmacology manifesting as electrophysiological differences in striatal projection neuron (SPN) spike rates, SPNs *should* increase spiking during the motor response to l-DOPA. In fact, stimulating specific subsets of these neurons to spike in freely-moving wild type and parkinsonian animals causes or inhibits motor activity as predicted. However, pharmacological effects of DA deficiency, let alone those of DA replacement, on SPN spiking activity in freely-moving animals are poorly studied and ultimately unknown. Showing the activity of SPNs of both in-/direct pathways may help elucidate mechanisms by which l-DOPA increases motor activity to normal and sometimes abnormal levels; such mechanistic information would advance understanding about how DA is such a potent motor stimulant.

To this end, I devised a Top-hat u-array (with microdrive) for recording in the striatum while stimulating the reticulated substantia nigra. Using my micro-array, I tested l-DOPA's acute effect on SPN spiking activity within contexts that varied in DA deficiency according to the *Pitx3*Null mouse's Parkinson's-like gradient of striatal DA denervation. Evidently, chronic DA denervation renders SPNs hyper-responsive to l-DOPA and a D1 agonist, SKF 81297, as indicated by exaggerated SPN spike rate responses biased by low baselines in *Pitx3*Null mice compared to wild-type mice. However, this may be a motor network effect on spiking as it was found in both dorsal (DA-denervated) and non-dorsal (having residual DA) *Pitx3*Null striatal regions. Furthermore, antidromically identifying dorsal SPNs allowed us to putatively distinguish a particularly relevant subset (striatonigral, D1-SPNs or d[irect]SPNs) known to elicit movement; serendipitously we also identified putative fibers of passage that strongly resembled striatal interneurons. D1-SPNs in *Pitx3*Null animals had baselines about an order of magnitude significantly below those in wild-type, and all increased firing more so in *Pitx3*Null than wild-type mice after drug injections, which lends some credence to the hypothesis that direct pathway SPNs are hyper-responsive during l-DOPA-induced normal and abnormal motor behavior secondary to DA depletion. Furthermore, they uncover a need to incorporate more neural factors in explaining electrophysiological effects attributable to DA denervation and restoration pharmacology. The latter data (putative fibers) tempt the interpretation that cortical axons of passage are being mistaken for striatal fast-spiking interneurons in the literature more often than not.

Document Type

Thesis

Degree Name

Master of Science (MS)

Program

Biomedical Sciences

Research Advisor

Fu-Ming Zhou, M.D., Ph.D.

Keywords

striatal, antidromic, electrophysiology, Pitx3Null, fast-spiking, dopamine, Parkinson's, spiny, freely-moving

Subject Categories

Medical Biochemistry | Medical Neurobiology | Medical Sciences | Medicine and Health Sciences |
Nervous System Diseases | Neurology

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Projection Neuron Responses to l-DOPA and D1 Agonism and Lack Baseline
Striatonigral Spiking**

A Thesis
Presented for
The Graduate Studies Council
The University of Tennessee
Health Science Center

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science (Under Protest)
From The University of Tennessee

By
Ben Sagot
December 2017

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DEDICATION

Strictly speaking this work is dedicated to the community that finds it most valuable. I do not expect my family, friends and romances to care much about this work, but they certainly empowered me to continue when it seemed that doing so might not be worth it.

My advisor, Fu-Ming Zhou, dissertation committee members, Kazuko Sakata, Jeff Steketee, Kristen O’Connell, and Wen-Lin Sun, and my past advisor, Detlef Heck, might benefit from perusing this work. They might not.

ACKNOWLEDGEMENTS

A double-handed thankyou to all my mentors: those who taught me at times from their knowledge (some people more often than others, Bob Waters) and at other times from their ignorance (some others more often than the rest...).

I got years of emotional, moral, technical help to continue this work; I need to thank those who eased the tensions of everyday life, most especially Momma Hen and Rooster Mike, old romantic roommie Lauryn Spielvogel (sometimes just in memory), Trusted Advisor Bill Veaser, others I met along the way that spiced up the entire experience: Eliza, Kyle, WeiWei, Qiu, LiLi, Sean, Andy, Afshin, Udita, Felisa, David, Ikbale, Hollyanne, Brittany, Natasha, Malik lab members (Nayaab, Ajeeth, Kamalika, Purnima, Chi-young, Ji-soo)...surely others I've forgotten here. Jackie inspired peace of mind I could never have had by myself without going the full nine on a whole 'nother degree, which made this entire transition out of grad school free of the regret of wondering what could have been if I had just put in more effort; I tried harder and longer than necessary.

But when I was losing myself and questioned everything about the process, I needed to reconnect with my purpose for continued sanity, and I discovered that the source of all the ancient Eastern wisdom I could ever want worked right next to me in the lab as a postdoc: my brother from an Asian mother, Shengyuan Ding. He taught me and we learned together how to care for relationships more than ideas; this may be the most lasting lesson I learn from my work on this project. It will be many years in the future and in very different contexts when I realize the myriad ways he enlightened our conversations and the full impact he had on my development, even more on my emotional maturation than as a scientist.

ABSTRACT

L-3,4 dihydroxyphenylalanine (l-DOPA) strongly stimulates motor activity in parkinsonian patients and animal models of Parkinson's disease. Severe striatal dopamine (DA) loss characterizes Parkinson's disease and its animal models. Given the canonical rate model of Parkinson's Disease pathophysiology based on differences in DA pharmacology manifesting as electrophysiological differences in striatal projection neuron (SPN) spike rates, SPNs *should* increase spiking during the motor response to l-DOPA. In fact, stimulating specific subsets of these neurons to spike in freely-moving wild type and parkinsonian animals causes or inhibits motor activity as predicted. However, pharmacological effects of DA deficiency, let alone those of DA replacement, on SPN spiking activity in freely-moving animals are poorly studied and ultimately unknown. Showing the activity of SPNs of both in-/direct pathways may help elucidate mechanisms by which l-DOPA increases motor activity to normal and sometimes abnormal levels; such mechanistic information would advance understanding about how DA is such a potent motor stimulant.

To this end, I devised a Top-hat u-array (with microdrive) for recording in the striatum while stimulating the reticulated substantia nigra. Using my micro-array, I tested l-DOPA's acute effect on SPN spiking activity within contexts that varied in DA deficiency according to the *Pitx3*Null mouse's Parkinson's-like gradient of striatal DA denervation. Evidently, chronic DA denervation renders SPNs hyper-responsive to l-DOPA and a D1 agonist, SKF 81297, as indicated by exaggerated SPN spike rate responses biased by low baselines in *Pitx3*Null mice compared to wild-type mice. However, this may be a motor network effect on spiking as it was found in both dorsal (DA-denervated) and non-dorsal (having residual DA) *Pitx3*Null striatal regions. Furthermore, antidromically identifying dorsal SPNs allowed us to putatively distinguish a particularly relevant subset (striatonigral, D1-SPNs or d[irect]SPNs) known to elicit movement; serendipitously we also identified putative fibers of passage that strongly resembled striatal interneurons. D1-SPNs in *Pitx3*Null animals had baselines about an order of magnitude significantly below those in wild-type, and all increased firing more so in *Pitx3*Null than wild-type mice after drug injections, which lends some credence to the hypothesis that direct pathway SPNs are hyper-responsive during l-DOPA-induced normal and abnormal motor behavior secondary to DA depletion. Furthermore, they uncover a need to incorporate more neural factors in explaining electrophysiological effects attributable to DA denervation and restoration pharmacology. The latter data (putative fibers) tempt the interpretation that cortical axons of passage are being mistaken for striatal fast-spiking interneurons in the literature more often than not.

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION	1
Aim 1: Chart the Effect of DA Agonists on MSN Firing Rates over Varying Levels of DA Deficiency.....	1
Aim 2: Chart the Effect of DA Agonists on Identified Striatonigral SPNs over Varying Levels of DA Deficiency	1
Aim 3. To Get Lucky and Find a Random Phenomenon That Upends My Understanding of FSI Identification	1
CHAPTER 2. LITERATURE REVIEW	3
Rodent-Based Research of Parkinson's Disease	3
Parkinson's disease and treatment	3
Basal ganglia neurophysiology: the canonical model.....	3
SPN neurophysiology in DA denervated conditions	4
Mistaking Fibers of Passage for Fast-Spiking Striatal Interneurons	5
CHAPTER 3. METHODS.....	7
Top-Hat U-Array: Design, Fabrication, and Use.....	7
Top-hat u-array design	7
Polytrode fabrication.....	10
Top-hat u-array fabrication	12
Registering the skull base to the connector (interface board) and feet	12
Drilling out and fitting microdrive components	14
Assembling the microdrives	15
Anchoring the connector interface board and connecting polytrodes	16
Gold-plating protocol.....	18
During surgery	18
Recovering Top-hat u-array parts	20
<i>Pitx3</i> Null Mouse Model.....	20
Recording Animal Motor Behavior before and after DA Agonism	22
Electrophysiological Recordings	22
Antidromic Identification of Single Units	24
A Note on Technical Differences Between the Two Studies	30
Statistical Considerations.....	30
CHAPTER 4. RESULTS.....	34
Results for DA Agonism in DA-Deficient SPNs.....	34
Basal SPN mean firing in WT and DA-deficient <i>Pitx3</i> Null mice	34
Response polarity differences among WT and DA-deficient <i>Pitx3</i> Null mice.....	34
l-DOPA response polarities	42
D1 agonist ersponse polarities.	42
Response intensity differences among WT and DA-deficient <i>Pitx3</i> Null mice	44
l-DOPA response intensities	46

D1 agonist response intensities	48
Antidromically identified SPNs are deficient in baseline spiking and respond strongly to DA agonism	48
Results for Fibers of Passage Mistaken as FSIs	50
CHAPTER 5. DISCUSSIONS	60
Discussion for DA Agonism in DA-Deficient SPNs.....	60
Interpretation within the Canonical Rate Model.....	60
Genotype differences	60
Baselines	61
l-DOPA responses.....	62
D1 agonist, SKF 81297.....	63
Striatonigral cell response intensities.....	65
Depth differences	66
In literature context	68
In defense of the causal D1-SPN hypothesis	69
Discussion of Striatal Fibers of Passage Being Mistaken for FSIs.....	74
Conclusions.....	74
LIST OF REFERENCES	76
VITA.....	89

LIST OF TABLES

Table 1.	Datasets Used.....	35
Table 2.	Antidromically Identified Cell Subset.	35
Table 3.	Baselines between Genotype and Depth.....	36
Table 4.	Overall Significant Results and Post-Hoc Comparisons for Both Drug Response Datasets.	43
Table 5.	Antidromically Identified Cell Baselines Between Genotype and Depth. ...	49

LIST OF FIGURES

Figure 1	Implanted Top-Hat U-Array.	8
Figure 2.	Feet Interface Microdrives with Base.	9
Figure 3.	Capture and Spinning of Polytrode Wires.	11
Figure 4.	Materials Useful in Top-Hat U-Array Fabrication.	13
Figure 5.	Pinning Electrode Wires to Interface Board.	17
Figure 6.	Gold-Plating Electrodes.	19
Figure 7.	Recovered Top-Hat U-Array Used for Parameter Checks after Months of Implantation.	21
Figure 8.	The Open Field Cage.	23
Figure 9.	Raw Data and Isolated Units.....	25
Figure 10.	Exemplary Dorsal WT Data under l-DOPA.	26
Figure 11.	Exemplary Non-Dorsal WT Data under l-DOPA.....	27
Figure 12.	Exemplary Non-Dorsal <i>Pitx3Null</i> Data under l-DOPA.....	28
Figure 13.	Exemplary Dorsal <i>Pitx3Null</i> Data under D1 Agonist.....	29
Figure 14.	Antidromic Spike Technical Theory.....	31
Figure 15.	Representative Antidromic Identification Criteria and Collisions.....	32
Figure 16.	Modelled Mean Baselines +/- Standard Errors for Each Group.	36
Figure 17.	Unexpected Responses to Selective D1 Agonism.	37
Figure 18.	Exemplary Dorsal <i>Pitx3Null</i> Responses to l-DOPA.	38
Figure 19.	Exemplary Non-Dorsal <i>Pitx3Null</i> Responses to l-DOPA.	39
Figure 20.	Exemplary Dorsal WT Responses to D1 Ag.	40
Figure 21.	Exemplary Non-Dorsal WT Responses to l-DOPA.....	41
Figure 22.	Response Polarity vs. Log Baseline.....	45
Figure 23.	Mean Post-Drug Responses Are as Log-Distributed as the Baselines.	47

Figure 24.	Baseline Data (Least-Squared Means) for Putative D1 Cells.....	51
Figure 25.	<i>Pitx3</i> Null Striatonigral Cells All Hyper-Responsive to Drug Compared to WT.....	52
Figure 26.	All Responding Units.....	53
Figure 27.	All Collisions	56
Figure 28.	All Neighboring Units and Two Co-responding SPNs.....	58

LIST OF ABBREVIATIONS

BG	basal ganglia
D1-SPN	D1 receptor-expressing, striatonigral, striatal projection neuron
D2-SPN	D2 receptor-expressing, striatopallidal, striatal projection neuron
DA	dopamine
FSI	fast-spiking interneuron
GP	globus pallidus
ISI	Inter-stimulus/-spike interval
l-DOPA	l-3, 4 dihydroxyphenylalanine
PD	Parkinson's Disease
SNc	substantia nigra compacta
SNr	substantia nigra reticulata

CHAPTER 1. INTRODUCTION

Aim 1: Chart the Effect of DA Agonists on MSN Firing Rates over Varying Levels of DA Deficiency

In order to contrast the spiking effects of DA agonists in the context of DA deficiency against those of WT mice and against the non-dorsal *Pitx3*Null (DA-sufficient) context, we will use extracellular single-neuron recording during freely-moving behavior.

- **Aim 1a.** To contrast l-DOPA's effect on dorsal SPN spiking in *Pitx3*Null mice against that of WT during free behavior. Preliminary data support expectations.
- **Aim 1b.** To contrast the effect of D1 agonist, SKF 81297, on dorsal SPN spiking in *Pitx3*Null mice against that of WT during free behavior. D1 agonism also accompanies increased normal and abnormal motor activity in this and other models of DA responsiveness in the DA deficient context (Li&Zhou, 2013; Bishop et al, 2005.)
- **Aim 1c.** To correlate SPN rate-increases with dorsoventral recording depth to show an effect of DA deficiency on SPN spike rates.

Aim 2: Chart the Effect of DA Agonists on Identified Striatonigral SPNs over Varying Levels of DA Deficiency

In an effort to show that most cells increasing firing during movement are indeed D1-SPNs, we will perform separate recordings mirroring those above and afterward identify putative D1-SPNs. Since D2-SPNs do not have excitable fibers in the substantia nigra reticulata, spikes antidromically elicited from that region putatively identify D1-SPNs as striatonigral.

- **Aim 2a.** To contrast l-DOPA's effect on dorsal D1-SPN spiking in *Pitx3*Null mice against WT during free behavior.
- **Aim 2b.** To contrast D1agonist's effect on dorsal D1-SPN spiking in *Pitx3*Null mice against WT during free behavior.

Aim 3. To Get Lucky and Find a Random Phenomenon That Upends My Understanding of FSI Identification

Striatal FSIs are routinely identified by their relatively high rate and narrow waveshape; however, the serendipitous discovery of their antidromic spiking evidence indicates that they might be fibers of passage.

- **Aim 3a.** To collate antidromic evidence for recording striatal fibers of passage; characterize said waveforms and their corresponding units.

CHAPTER 2. LITERATURE REVIEW

Rodent-Based Research of Parkinson's Disease

Parkinson's disease and treatment

At autopsy, Parkinson's Disease (**PD**) patients show an especially marked degeneration of the nigrostriatal fibers that supply the striatum with dopamine (**DA**). (Kish et al. 1987 or 1988; Hornykiewicz 2001; Kowdoer et al. 2013). This otherwise intense striatal DA innervation (from the compact substantia nigra) complements heavy striatal DA receptor expression (Levey et al 1993; Yung et al 1995). Striatal DA loss invariably precipitates hypokinesia once it reaches ~80% (Kim et al., 2000 see also Fig. 4 from Schwarting and Huston, 1996) and is proposed to induce a hypersensitivity to dopamine (Cenci & Konradi, 2010). After DA depletion, DA agonism elicits cellular markers (e.g. ERK, delta/FosB) of supersensitive dopamine D1 receptor stimulation (Gerfen et al, 2002; Pavon et al, 2006; Corvol et al, 2004) as it stimulates increases in both ab-/normal motor activity (Francardo & Cenci, 2014; Li et al, 2015).

The most common PD treatment is DA replacement therapy using l-3,4-dihydroxyphenylalanine (**l-DOPA**) because of its efficacy in stimulating motor activity (Olanow et al, 2009). In addition to normal motor activity, it may eventually induce hyperkinetic side effects, known as l-DOPA-induced dyskinesias (Olanow et al, 2009, Bastide et al., 2015). During l-DOPA's effect on behavior, it affects DA deficient striatal information processing, but this is poorly characterized (Iversen, 2010, pg 438), which is one striking reason for a continued focus on the behavioral and physiological manifestations of DA pharmacology in the context of DA loss (Olanow et al, 2009).

Basal ganglia neurophysiology: the canonical model

The striatum, a key component of the basal ganglia (**BG**) motor control circuit, is thought to be directly responsible for the motor symptoms of PD and of PD treatment (Hornykiewicz 2001; Franco & Turner 2012). The striatum is the BG structure that processes the majority of the BG's synaptic input, which is sent downstream via two distinct output pathways. One is the direct pathway in which dopamine D1 receptor-expressing striatal projection neurons (**D1-SPN**, a.k.a. D1 striatal spiny projection neuron, D1-SSPN, D1 medium spiny neuron, D1-MSN, and dSPN) can ultimately activate movement via spiking. The other is the indirect pathway in which dopamine D2 receptor-expressing SPNs (**D2-SPN**, etc, and iSPN) can ultimately inhibit movement via spiking (Kravitz et al, 2010). These downstream effects of spiking on behavior operate in both intact and DA-lesioned mice (Kravitz et al, 2010).

Mechanistically, these phenomena are interpreted most often via the canonical rate model of the BG explained thus: both cell types release GABA but have opposing

effects due to the nuclei into which they synapse. Direct pathway SPN spikes *disinhibit* thalamocortical motor networks (allowing behavior) by directly inhibiting BG output nuclei, e.g. the substantia nigra reticulata (SNr) (Kravitz et al, 2010; Freeze et al. 2013). Conversely, indirect pathway SPNs indirectly *inhibit* thalamocortical motor networks, by synapsing on intermediate inhibitory BG nodes; synapsing into these nodes reverses the polarity of their effect on downstream neuronal, and ultimately behavioral, activity. By way of example, specific D2-SPN activation has been shown to cease movements (Kravitz et al, 2010, Sano et al.,2013); this is interpreted canonically as inhibiting an intermediate inhibitory nucleus, which disinhibits the (inhibitory) SNr ultimately rendering thalamocortical motor nuclei further inhibited and reducing movement. Overall then, increases in D1-SPN spiking promote movement via the direct pathway, and increases in D2-SPN spiking inhibit it via the indirect pathway.

SPN neurophysiology in DA denervated conditions

Understanding what role stimulation of DA receptors on SPNs plays in their spiking might reveal mechanisms underlying l-DOPA's cell-level effects that accompany motor activity and could have a bearing on the interpretation of DA's function in the normal and pathological BG. Published results are interpreted to affect spiking indirectly through networks and/or are inconsistent. For example, in vitro SPN recordings from DA-lesioned animals are generally interpreted in terms of effects on corticostriatal glutamate release (reduced by aberrant D2 signaling after DA denervation and rescued by l-DOPA; Picconi et al, 2004) and long-term synaptic changes (aberrant Ca^{2+} currents after DA depletion; Prieto et al, 2009), whereas the canonical model is more directly invoked to explain in vivo anesthetized results in terms of spike output (increased background spiking after DA depletion normalized by l-DOPA; Kita&Kita, 2011a, b). However, the fact that anesthetized (and in vitro) SPNs show drastically different spontaneous firing properties and membrane potential states than in awake animals (namely, less variable rates and bimodal resting membrane voltage; Mahon et al, 2006; Kish et al, 1999; Rebec, 1998) highlights the importance of behaving animal recordings in exploring the effects of DA denervation on SPN spiking during behavior. Despite the seemingly straightforward connection between direct stimulation of D1-SPNs causing their spiking and consequent motor activity (Kravitz et al, 2010) and supersensitive DA receptors (which SPNs likely express more heavily than any other brain area due to their baseline expression being many-fold higher; see e.g. Fig. 2 of Delis et al., 2013) possibly being one cause of LID (Cenci & Konradi, 2010), effects of DA agonism on DA-deficient SPN spiking during such behavior is poorly researched. Such recordings are rare in primates (not one according to the following review: Boraud et al., 2002, at least one thereafter: Liang et al., 2008, Singh et al., 2015, 2016) and rare and conflicting in rodents (Iversen, 2010, pg. 438; Kish et al, 1999; Chen et al, 2001). Nonetheless, knowing these receptor and cell level events is necessary if one wants a full account of BG functionality to explain l-DOPA's behavioral effects in enough mechanistic detail to refine understanding of how SPN activity informs behavior.

Since specifically D1-SPN rate increases induce motor behavior (in both DA un-/lesioned conditions; Kravitz et al, 2010), and DA denervation of striatal cells sensitizes the BG to do the same under l-DOPA (thus DA) agonism (Corvol et al, 2004; Cenci and Konradi, 2010), then it is expected that at least part of the underlying mechanism of motor activity induction by l-DOPA would be the rendering of D1-SPNs hyper-responsive to DA agonism by DA denervation (Feyder et al., 2011, Iversen, 2010, pg. 438, and Murer & Moratalla, 2011).

To this end, we recorded SPN responses to l-DOPA and D1 agonist, SKF-81297 in DA deficient (*Pitx3*Null dorsal striatum, defined further in Methods) and DA sufficient (*Pitx3*Null non-dorsal and WT dorsal striata) conditions. We further identified a subset of striatonigral SPNs antidromically in order to begin interpreting the results from the in-/direct pathways perspective.

The *Pitx3*Null mouse models l-DOPA-induced motor activity. This is because it has a chronic, spatially restricted, dorsoventral DA deficiency gradient and a robust ab-/normal motor response to l-DOPA that is absent in WT animals (Francardo & Cenci, 2014; Hwang et al, 2005). Contrasting these against WT mice, we looked for electrophysiological evidence consistent with the predominant hypothesis (see e.g. Cenci and Konradi, 2010 and Murer & Moratalla, 2011) that l-DOPA induces motor activity via rate increases in D1-SPNs. By testing SPN responses to DA agonism, we expected to discover a spiking mechanism putatively underlying LIM. Specifically, we looked for any evidence that DA deficiency would be associated with a change in baseline rate between groups before we tested the hypothesis that DA-denervated SPNs (i.e. from dorsal *Pitx3*Null mice striata) show exaggerated spiking responses during their DA-agonism-induced motor activity superseding those of dorsal WT SPNs (fully DA innervated). In addition, we tested the hypothesis that dorsal *Pitx3*Null SPNs increased spiking in response to DA agonism more so than non-dorsal *Pitx3*Null (less DA denervated) SPNs. Finally, we re-tested the first hypothesis in a subset of antidromically identified SPNs.

Mistaking Fibers of Passage for Fast-Spiking Striatal Interneurons

Electrophysiologically recording the striatal neurons of rodents is a commonly performed technique in the neuroscience literature concerning normal and diseased brain function (e.g. Parkinson's disease, drugs of abuse). (Gittis et al, 2011, Kubota et al, 2009, Lee et al, 2017, o'Hare et al, 2017, Sagot & Zhou, submitted) Aside from the inherent technical difficulties of electrophysiological recordings, focusing research questions on a particular subtype of neuron further decreases the likelihood of sampling from a population of interest. According to many publications (Graveland & Difiglia 1985, Saka 2002, Rymar et al 2004), interneurons represent <5% of total striatal neurons, which can further be broken down into different interneuron types; e.g. tonically active neurons (TANs), and fast-spiking interneurons (FSIs), also known as parvalbumin-positive (PV+) interneurons. Luk and Sadikot, 2001, report that PV+ interneurons make up only 0.7% of striatal interneurons. Thus attempts to record these would at least initially be expected to

have much fewer opportunities to do so and be reflected in unbiased samples at approximately the same rate.

However, in vivo electrophysiologists have reported FSI proportions in striatal samples to account for 7-30%. (Barnes 2005, Kubota et al, 2009, Hernandez et al, 2013, Berke et al 2004 and 2008, Gage et al, 2010, Schmitzer Torbert and Redish 2009, Lee et al, 2017) When unintentional (as opposed to e.g. Mallet, 2005), this obvious discrepancy has been hypothesized to result from their biased distribution towards the dorsolateral striatum (Berke 2011) and a general facility for recording data from neurons having larger somata and higher spike rates due to their more obvious presence during spike sampling before recording. (Heinricher in *Microelectrode Recording in Movement Disorder Surgery*, 2004 ed.)

Identification metrics in these cases have used primarily waveshape, and some combination of firing rates, ISI histograms, autocorrelograms, or peri-event rasters (Barnes et al, 2005, Mallet 2005 and 2006, Kubota 2009, Schmitzer Torbert and Redish 2009, Tepper, 2010, Gittis et al, 2011, o'Hare et al, 2017), but these criteria are inconsistently seen, reflecting the lack of knowledge over clearly distinguishing FSI traits in vivo; unambiguous identification is rare (Tepper and Koos, 2017). Thus resolving the identity of these units potentially stands to shed new light on previous conclusions, e.g. the task-relevance of FSIs (Berke et al., 2004, Kubota et al, 2009, Tepper & Koos, 2017)

Some hint of their identity came accidentally when, during antidromic identification of direct pathway SPNs, I recorded antidromic responses in the striatum having the waveshapes and ISI characteristics much more commonly associated with FSIs. Other evidence supports this interpretation: response latencies predicted by theoretical expectations of myelinated axons (Jinnai & Matsuda, 1979) and putative fibers clustered with up to 4 others (as reported: Berke, 2008) These tempt the interpretation that these are better explained as cortical fibers of passage, however more research should confirm this hypothesis.

CHAPTER 3. METHODS

Top-Hat U-Array: Design, Fabrication, and Use

In order to record neural activity from freely moving animals, a device must effectively host the interface between the brain and the recording equipment, must minimize interference with the animal's behavior (most likely being lightweight, ~10% of body weight) and be able to withstand months of implantation on the animal's head, and –if the neural coverage is not sufficient– must reposition electrodes in order to optimize recordings as well. Usually this device is referred to as a microarray, with or without a microdrive for repositioning electrodes.

In order to accomplish these using as few and inexpensive resources as possible, I designed a microarray I call the “Top-hat u-array” due to its appearance on the animal once implanted. **(Figure 1)** The design, fabrication, and use of this microarray are described here.

Top-hat u-array design

In designing the array, considering the brain areas from which one will record or stimulate forces the device to have certain parameters since volume and component proximity are limited; in our case, the simultaneous recording/stimulation (using electrode sets inside stabilizing tubes having < 0.26 mm. outer diameter) from a maximum of four locations having inter-site spacing > 0.5 mm. were made possible in the rostral cortex, striatum (str), and substantia nigra pars reticulata (SNr) of one hemisphere of a mouse. Flexibility of the design for starting materials is maintained by using small –compared to the entire available volume–, modular pieces (e.g. tubes, screws, springs, etc. as opposed to 3D printed parts). Flexibility for accommodating other brain areas and hemispheres is maintained by pre-registering the top hat's components – with respect to one another and ultimately to the skull–, i.e. 1:1 placement of array components into a base that doubles as a “skull map” (a common frame of reference) without having to place everything relative to each other during surgery. Flexibility in the dorsoventral positioning of electrodes is maintained by including a microdrive mechanism, which in our case was done by registering another skull map on top of the first in order to subtractively form “feet” that interface between the drive screw and the carrying tube. **(Figure 2)** showcases a completed Top-hat minus debris shield and ground wire.)

A miniature connector and interface, or breakout, board for connecting electrodes to the input connector pins and ultimately to the recording system was in our case essential in terms of minimizing weight, facilitating input connections, and facilitating registration to the stereotax during implantation. The EIB-16 from Neuralynx we used might be unable

Figure 1 Implanted Top-Hat U-Array.

Upper left and upper middle – the implantation steps preceding recovery during ground wire placement. Upper right – the animal grooming its paws after recovery. Lower left – the animal roaming its cage after recovery. Lower right – close-up of implant highlighting placement with respect to skull, dental cement anchoring to screws, windows in debris shield, and translucence

Figure 2. Feet Interface Microdrives with Base.

Upper left – an assembled microdrive set with connector anchored to base. Upper right – shield tubing during placement (although this step is normally done after electrode placement, gluing, and pinning): window seen between thumb and forefinger on right. Lower left – preparing to insert polytrodes into carrier tubes. Lower middle and right – close-ups of assembled microdrive set and anchored connector (stimulating electrode set clearer although its slight protrusion is problematic due to fragility of fine tips)

to be improved upon, and in our case doubles as a cover for the Top-hat u-array, keeping most environmental debris out for longer than other arrangements. (Furthermore, they were re-usable at least once, if not multiple times until obvious defects arose or the connector did not hold its mate >5 minutes during animal behavior.)

Similarly, in such an arrangement, a transparent and durable shield should protect the mechanisms and wiring; in our case, clear, cylindrical, plastic tubing (metallic wire mesh may also work to a slightly lesser extent and allow for electromagnetic shielding when attached to ground) that fits snugly around the entire contraption resting atop the base is essential for excluding debris and interference from the animal's movement. However, the ability to insert tools for troubleshooting through this wall comes at the cost of excluding debris (but can facilitate rinsing if appropriately situated), so optimizing the size and placement of windows in the shield should be carefully considered. (**Figure 2**, upper right)

Polytrode fabrication

Polytrodes (electrodes wrapped together in various numbers) are necessary in order to complete the array; fabricating them before the microarray affords an economy of scale (provided the protocol yields acceptable work-product) since they are less labor intensive (and thus easier to make in bulk) and because having them available allows one to focus on finishing the microarray once begun.

The protocol of Liao et al, 2011, was adapted to our purposes (since we used ~12.5 um nichrome wire from Kanthal Palm Coast, Palm Beach, FL); specifically starting from the proposition that 4 wires having 25 um outer diameter can withstand 60 turns/cm without failing, and assuming a linear relationship between the number of turns and the number and diameter of wires to be included in the polytrode, then twice the number of wires at half the diameter should yield the same acceptable turn ratio. An intuitive way to consider this approximation is that one could theoretically cut each 25 um wire into two 12.5 um wires without disturbing too much the turn ratio; thus, eight ~12.5 um wires should withstand about 60 turns/cm. (**Figure 3**)

(More electrodes than four were used per polytrode since preliminary results showed non-orthogonal –highly correlated– data on four non-splayed electrodes, i.e. overlapping data, rendering tetraode utility comparable to a single electrode. At times 8 and sometimes 6 wires were used, which helped splay some wires from the others and allowed us to use only the most orthogonal data during spike sorting. Incidentally, introducing splay manually had a similar though less reliable effect, although splaying should generally not precede insertion of the polytrode through its stabilizing or carrying tube, i.e. the final steps.)

Figure 3. Capture and Spinning of Polytrode Wires.

Upper left and right – capture of wetted polytrode wires within polyethylene-tipped hemostats. Lower left – hemostats suspended above magnetic stirrer on lowest spin setting (~ 1 rev./sec). Lower right – hemostats set held in polyethylene tubing loop (away from stirrer) unspinning freely until rest

Taking the number of wires to be turned together into the polytrode was done by folding an extended piece of nichrome as many times as necessary to create the desired multiplicity at ~15 cm length (i.e. ~90 cm for a 6-trode, 120 for n 8-trode), then running the bunch under a stream of water while pinching the tip with more folds (i.e. leaving one tip loose to be straightened uniformly by the water); once wires stayed in a smooth bundle, this pinched section was transferred carefully to straight-tipped forceps to be hung by its handle on two appropriately spaced and sized knobs. Bundling the wires at the other tip may require another run under water, but once both tips are held each by straight-tipped forceps (one pair of which is hooked onto the knobs), then a magnetic stirrer is placed underneath the lower pair and centered with respect to it. The turn ratio then translates well into the rate at which the polytrode is spun: 1 minute/cm for turn ratios of 60/cm.

Once the polytrode has the desired number of turns, the magnetic stirrer is removed and the polytrode allowed to un-spin and re-spin until coming to rest (**Figure 3**, lower right), at which point it is safe to remove the forceps and store the polytrode somewhere that dust and static are minimized (for ease of array fabrication).

Top-hat u-array fabrication

Registering the skull base to the connector (interface board) and feet

A picture and description of parts used in the Top-hat u-array is found in **Figure 4**.

(In order to facilitate fixture, before gluing, of components into the plastic, drill bit diameters should be rated for widths at least 10-15um smaller than the outer diameter of the tube expected to be placed through the resulting hole, e.g. 0.25 mm. for a 31 ga (0.26 mm outer diameter) tube such that insertion is smooth yet temporarily held in place without gluing.)

In the first step of Top-hat u-array fabrication, two polycarbonate pieces of sufficient size to accommodate the footprint of the shielding tube (~2x2 cm) are registered to each other by means of two holes simultaneously precision drilled (MicroLux, Micro-Mark, Berkeley Heights, NJ) at opposite corners of both layers—one layer on top of the other— and inserting appropriately sized rigid tubing until flush with at least one layer of the two.

(Measuring exact locations of sites on this two-layered piece can be difficult without having either markings in the microdrill's retaining bars or preset markings on the piece itself; we used the former in order to avoid repeating the latter for every Top-hat.)

Figure 4. Materials Useful in Top-Hat U-Array Fabrication. Upper left – precision drill press and guide bar (having metric notches unseen here). Upper middle – dissection microscope and fabrication platform having lighting. Upper left – stimulus isolator, impedance meter, and plating station. Lower left – precision tools and consumables. Lower right – microdrive and polytrode consumables.

Marking the anteroposterior (AP) and mediolateral (ML) locations of desired recordings is straightforward; define a center of the base (perhaps as bregma or lambda or some variant), then take each coordinate as an ordered pair $-(x, y)$, where x = ML or AP coordinate, and y = the respective other— then carefully drill through both layers of polycarbonate using the drill bit corresponding to the outer diameter of the smallest carrying tube (in our case 0.25 mm, corresponding to the 31 ga tube). The resulting holes will eventually accommodate the carrying tube in the top layer and mark the guide tube location in the second layer.

(Using smaller drill bits before larger in the fabrication process mitigates the impact of some drilling errors.)

Without turning the piece over or separating the layers, drill eight holes appropriately sized (in our case, ~ 1.0 mm) to fit the drive screws (in our case, 00-90 screws, 1.194 mm outer diameter) in an equilateral octagon around the center of the base, 4 of which will be covered by interface board corners—2 of those being used for screwing the board to the base— and 4 of which will anchor the carrying tubes to the base and act as an actuator for the microdrive mechanism. The distance from the center to the screw holes should equal half the distance between the two screws that will anchor the interface board (in our case the two screw holes at opposite corners of the EIB-16, which have a center to center distance ~ 12.5 mm).

Drilling out and fitting microdrive components

Separate the layers; choose a top layer—for making feet that connect the carrying tube to the drive screws— and a bottom layer—as the base to hold guide tubes, anchoring screws, drive screws, and the shielding tube.

The next largest drill bit (in our case, sufficient to snugly fit a 26 ga guide tube, ~ 0.45 mm, for polytrodes and to fit a 20 ga for the stimulating electrode guide tube, ~ 0.9 mm) is used to drill out the marked guide tube sites on the chosen bottom (base) layer. Drilling an extra hole within the perimeter will allow a ground wire (otherwise passed down around the edge of the base) to be passed directly through the base and provide another anchor point for dental cement during implantation. Guide tubes may then be glued having the desired protrusion from the base being careful to ensure that they are as parallel as possible to each other.

On the same layer, a tap for the drive screws is used to carve into the 8 perimeter holes. These screws should be long enough (~ 25 mm in our case) to protrude from the bottom of the base (by ~ 6 -8 mm in our case) in order to provide anchoring to the skull using dental cement. Although not all 8 need be used (accommodating up to 4 drive screws + 2 anchoring screws + optional other anchoring screw projections from base), whichever are used varies primarily according to the ease with which certain feet connect to the carrying tubes (in our case, the EIB connector orientation was necessarily also

considered). The final step on the base layer is to align guide tubes with respect to each other.

As for the top layer, the primary concern is ensuring that whichever 4 (or fewer) of the 8 screw holes used to accommodate the drive screws, which hold the carrying tube feet, are designed and then fabricated without error; minimizing the distance between the drive screw and its carrying tube will ultimately maximize the downward force of the screw on the carrying tube, minimizing the chance to get stuck. Since it is much easier to drill out each drive screw hole than form the feet, the largest drill bit (in our case, 1.25 mm) is used to drill out all 8 perimeter holes, and 4 (or fewer) feet are then designed by marking boundaries between them –e.g. using a fine-tipped felt pen or scalpel tip– then formed subtractively –i.e. by removing surrounding shards of plastic at first using diagonal pliers then by a razor under a dissecting microscope. It is critical here to carefully remove as much plastic as possible from around the carrying tube hole (in order to keep it from interfering with others during use on the animal) without risking the integrity of the hole. The area around the drive screw holes requires similar care since they may otherwise interfere with shield tube placement at the final step.

(In either case, when a tube hole is compromised, superglue may carefully be applied either shortly thereafter or in the next step during the process of positioning carrying tubes inside guide tubes and affixing them to the feet, although be warned that superglue in such a confined space can easily cause more harm than good in such a delicate situation.)

Assembling the microdrives

Assembling the feet (once they are approximately shaped enough to be assembled) into the drive mechanisms can minimize damage to the feet during the final touches of their formation since they otherwise become much more difficult to hold once removed from one another. In order to assemble the feet, thread a drive screw (in our case, 3/8" 00-90 screw) downward through the foot's drive screw hole and into a spring. Then using a fingertip to steady this combination on the tip of a screwdriver (approximately upside-down), begin screwing this screw into the drive screw hole on the base, i.e. that has been tapped. Perform the same for the remaining feet and drive screws/springs unless they interfere with each other enough to preclude removing further shards. (**Figure 4**, lower right, circled)

Once the base holds all the microdrives, remove shards from the feet until they no longer interfere with each other upon use; inserting the carrying tubes through their holes into the guide tubes and screwing the drive screws as far as possible into the base can hold the feet in place to facilitate such removal.

(Replacing gloves when handling the fine gauge tubes minimizes the chance that debris will block the tube during polytrode or stimulating electrode insertion.)

Once the carrying tubes have been inserted into the guide tubes through their foot holes, they should be checked for patency using thin wire (in our case $\sim 0.05\text{--}0.1$ mm), and then glued to the feet while ensuring that their maximum extension and retraction do not interfere with the interface board or protrude too much. (In our case, guide tube patency was checked before precisely inserting the stimulating electrode set itself into the foot under the dissection microscope; carrying tubes were pre-pared in our case at 15 mm long to fit 10 mm long guide tubes and a presumed 17 mm EIB height) Ensure that at maximum extension the carrying tube does not exit the guide tube longer than $\sim 0.3\text{--}0.5$ mm, the approximate depth of dura from the top of the skull. Applying some superglue around each carrying tube on top of its foot mitigates the risk that it will slide within its hole during drive screw retraction, but doing the same underneath the foot risks fastening the carrying tube to the guide tube, so this latter step is best done with the drive screw fully retracted.

Anchoring the connector interface board and connecting polytrodes

Next, the connector interface board can be anchored to the base. First threading the anchoring screw (in our case, 1" 00-90 screw, Fasteners and Metal Products Corp., Waltham, MA) through a nut, then the board, then another nut allows one to tighten the nuts around the board so that movement of the board in its final situation is minimized. Once this is done for one side of the board, that anchoring screw is threaded into its tapped hole in the base.

Threading the second screw begins the same as the first, but the lower nut should only be tightened after this screw has been threaded into its anchoring hole (otherwise the screw might not tighten into the base but against the nut); serrated tweezers around the nut and a screwdriver to hold the screw in place are helpful here. More important in this step is the final height of interface board on each screw, which should be an equal distance from the base on both anchor points in order to minimize torsion on the connector board.

Polytrodes are then inserted into their carrying tubes (or stabilizing tubes first if necessary: in our case polyimide tubing, Part No. MIL 38, Microprobes, Gaithersburg, MD) and extended sufficiently from the guide tube in order to allow ~ 1 mm of the entire tip to be splayed before being dipped in ethyl cyanocrylate (Jog et al, 2002) and dried for the final cut exposing the electrode wires uniformly. Finally, taking the guide tube tip as skull depth, the cut polytrode is lowered as far as possible in order to define the furthest ventral location from which to be recorded unless the highest point to be recorded is more critical. In either case, the carrying tube is not yet glued to its contents –nor, ideally, are the stabilizing tubes glued to theirs– before connecting all individual electrode wires (except ground and reference) to the connector board and checking for connectivity during gold-plating or waiting until gold-plating is complete. At this point the electrode wires are individually pinned to the interface board; a gentle tug on each wire after pinning ensures that it has not broken at the point of contact. (**Figure 5**)

Figure 5. Pinning Electrode Wires to Interface Board. Upper left – capturing polytrode for insertion into carrying tube. Upper right – inserting gold pin into interface board receptacle having wire inserted. Lower left – pinching gold pin to wire and into receptacle. Lower right – completed Top-hat u-array showing all wires and tubes finally situated.

Gold-plating protocol

The gold-plating protocol essentially mirrors those in common use, but one extra step is borrowed from Ferguson et al, 2009 (whose protocol is otherwise exemplary): the addition of plating inhibitors in order to ultimately accomplish a “rice-like” texture on the electrode tip that reduces impedance without increasing site-exposure, i.e. increases sensitivity without sacrificing selectivity. Briefly, 1.125 mL of 1g/L PEG-8000 and 0.375 mL non-cyanide gold-plating solution (gold-plating solution, Neuralynx, Bozeman, MT) are combined in a 1.5 mL centrifuge tube. In our case, a complementary connector having individual pre-connected output wires accessible for running current through each electrode individually is affixed to the interface board’s connector, the polytrodes are all lowered into the solution. The electrode tips are first “cleaned” (metal oxides reduced and some debris repelled) by passing the opposite current as used for plating, i.e. electrode being positive, using 0.1 uA (controlled by a stimulus isolator: in our case, WPI 365) for < 5 seconds. Next, the polarity is reversed, and plating proceeds through all electrodes in succession using the same current (0.1 uA) for up to 30 seconds during the first round and for progressively shorter durations thereafter until electrode impedance measured ~50-100 kOhm; this often required longer duration pulses during successive rounds than those given in the reference. (**Figure 6**)

Spritzing with alcohol will not ruin the tip and was used as an antiseptic just before surgery (checking the need for re-plating beforehand if waiting > 1 week for surgery).

During surgery

In order to ensure accurate placement into final coordinates, registering the array to the stereotaxic apparatus is essential. Without having to assume the entire array has been made with right angles in mind at every fitting and gluing step, it was useful in our case to simply use the connector as an anchor point to the stereotax’s holder arm and make slight adjustments there that optimized the colinearity of the guide tubes with respect to the arm (which assumes the guide tubes have been aligned with respect to each other), e.g. by holding the arm against a straightedge and comparing the guide tubes to the same edge. The anchor point consists of a customized screw-in extension to the holder arm that clamps onto the connector via a screw-on faceplate; although the screws must be tightened as little as possible, the force holding the Top-hat u-array should not be overcome by gentle tugs on the ground wire. (**Figure 1**)

After preparing the animal for implantation following standard rodent surgical procedures –specifically after accurate formation of trephine holes for all necessary components (2 or 3 skull screws + up to 4 polytrode holes)–, the pre-registered array/arm combination is affixed to the stereotax, carefully lowered until guide tubes are ~1 cm above the skull, and sterile petrolatum or antibiotic ointment applied to each polytrode base at the guide tube (in order to prevent the entry of dental cement later). The main

Figure 6. Gold-Plating Electrodes.

Styrofoam holding plating solution in centrifuge tube, impedance tester connected to wire in solution (green lead) and individual electrode (colored mating connector wires wrapped around stand arm), changing polarity on right to plating (from cleaning)

ground wire is then wrapped around a skull screw –as far from the polytrodes as possible in order to facilitate later work with them– allowing some slack in order to enable slight retractions in case polytrodes need to be slightly repositioned.

In order to facilitate polytrode entry into the brain, it may be necessary to cut dura; in our case, the smallest syringe tip available having been slightly curved at the tip was used to pierce and retract dura, which requires careful monitoring of bleeding (and use of epinephrine and/or gelfoam and saline flushing / sterile swabbing as necessary). Taking note of the position on the stereotax ruler as each polytrode reaches the dura during penetration allows one to reconstruct (using the final position measurement) the depth that each polytrode has been placed below dura. Dental cement can then be draped from each guide tube at its protrusion from the base of the Top-hat u-array to its situation on the animal's skull, and liberally applied to all skull screws and any available anchoring screw tips; ensuring that all locations having cement eventually connect via cement to all others reinforces the integrity of the entire interface. Allow the dental cement to harden to the touch before unscrewing the faceplate that holds the connector as gingerly as possible and returning the animal to a recovery cage. No special precautions are necessary for the animal's wellbeing in the new cage except that it should be housed alone.

Recovering Top-hat u-array parts

Figure 7 pictures the Top-hat u-array after removal from a sacrificed animal. It is advisable to recover parts that are hard to find and/or expensive (in our case, the EIB-16 and 1-inch screws). Pliers can easily remove most large chunks of dental cement that has not been soaked in methyacrylate for softening. Smaller chunk in the grooves of screws (and large chunks that threaten the integrity of fragile parts) can be softened or carefully removed using finer tools.

It is also advisable to inspect the polytrode tips and protrusions (e.g. to inform depth estimates taken during recording), then unscrewing the microdrives –upward– to minimum protrusion, taking a measurement, and comparing to original parameters in order to check for evidence of drive, glue or tube failure before screwing downward to maximum protrusion for same.

***Pitx3*Null Mouse Model**

*Pitx3*Null mice lack a functioning *Pitx3* transcription factor, which ultimately leads to the death of the vast majority of SNc tyrosine hydroxylase positive (i.e. DA producing) neurons and DA receptor supersensitivity in the striatum (Hwang et al, 2005), while some VTA DA neurons are independent of *Pitx3* and thus survive (Jacobs et al, 2007; Hwang, 2003). This loss of DA neurons that would otherwise project to the dorsal striatum yields a dorsoventral DA denervation gradient in the striatum having the following

Figure 7. Recovered Top-Hat U-Array Used for Parameter Checks after Months of Implantation.

Upper left and right – differing angles of whole Top-hat u-array pulled from skull after sacrifice Middle left – 10x magnification of stimulating electrode set tips Middle right – 40x magnification of stimulating electrode set protruding from guide tube Lower left- 40x magnification of stimulating electrode set tips Lower right – 40x magnification of polytrode tips protruding from base (splay only slightly larger than when implanted), reference wire accompanies lower polytrode deinsulated to 1 mm from tip.

characteristics: relative to WT siblings, the nucleus accumbens (ventral striatum) retains ~40-80% of its releasable DA from ~30% of the midbrain DA-ergic axons; however, the dorsal striatum retains ~0-10% of its releasable DA from DA-ergic terminals totalling <10% of WT terminals (Smits et al 2007, Li & Zhou, 2013, Wei et al. 2013). This denervation gradient is associated with molecular markers of DA receptor supersensitivity in the dorsal striatum subsequent to l-DOPA in common with similar animal models (Francardo & Cenci, 2014).

Recording Animal Motor Behavior before and after DA Agonism

An animal protocol guiding this work was approved by the University of Tennessee Health Science Center's Laboratory Animal Care Unit's Institutional Animal Care and Use Committee. Male *Pitx3*Null and WT mice aged 6 (+/-4) months were used for all experiments as follows. A mouse was recorded beginning ~ 1 week after microdrive implantation while tethered to a commutator via lightweight headstage cable (Omniplex, Plexon Inc., Dallas, TX). A baseline saline injection was given 10 min after recording began, and the animal was allowed to explore the ~8"x8"x8" cage (having no top, see **Figure 8**). After ~ 80 min. had passed from recording start, l-DOPA (25 mg/kg with 5 mg/kg benserazide) or D1 agonist (1.0 mg/kg SKF 81297, higher doses tended to lead to epilepsy in *Pitx3*Null animals; this dose also minimized WT responses without going below our known significant responses in *Pitx3*Null mice from Li & Zhou, 2013) was injected, and 160 (l-DOPA) or 80 (D1 agonist) min were allowed to pass before the recording ended or another drug was delivered (for qualitative comparison). Doses for quantitative comparisons were given ~24 h apart from each other.

Electrophysiological Recordings

On each animal a microdrive array containing 16 Au-plated-nichrome electrode channels in various combinations of poly-trodal configuration (bundles consisting of between 4 and 8 wires) was implanted. A stimulating electrode was included in some microdrives for stimulating antidromic spikes from the substantia nigra reticulata in order to identify striatonigral SPNs. Surgery was performed under ketamine-xylazine (90 mg./kg. ketamine +10 mg./kg. xylazine in 1uL/g. .9% saline) anesthesia following standard procedures for rodent survival surgery (e.g. Richter et al, 2013).

Raw recordings were band-pass filtered in the acquisition software (Omniplex Server, Plexon Inc., Dallas, TX) 100 - 8000 Hz. Units were detected and classified by their threshold energy. Initially, putatively single unit clusters in various 3-dimensional metric spaces (e.g. using principal components, non-linear energy, time slices, etc. in Offline Sorter v3, Plexon Inc. Dallas, TX) were used to make unit templates, which were used as the basis for template-based sorting of remaining waveforms. Previously published criteria utilizing auto-correlations (not shown), inter-spike interval histograms, and spike waveshape were used for classifying units as SPN, or non-SPN (e.g. Mallet et

Figure 8. The Open Field Cage.Placed in front of a mirror inside a hollowed out filing cabinet, an 8"x8"x8" clear plastic cage having no top served as the mouse's open field. A cap of water is in the middle of the cage at the moment, although this was not routine. See text for details.

al, 2005, Schmitzer-Torbert and Redish, 2008). (**Figure 9**) All publication quality SPNs were characterized by ellipsoids of 1.96 standard deviations (as set in Offline Sorter) being out of contact with the nearest unit ellipsoids in a choice feature space (usually principle component space), having <0.1% of spikes violating a refractory period of 2.0 msec, and >90% of spikes recovered (inferred by comparing the distribution of valley values to an expected gaussian distribution; data not shown). An exemplary recording, units, and ID metrics are shown in **Figures 10-13**.

Antidromic Identification of Single Units

In order to ascertain the role of D1-SPNs here, they were putatively electrophysiologically identified in the hopes of finding them among those increasing activity (consistent with the canonical rate model) and to look for any evidence that their activity opposed expectations. Striatonigral (D1 receptor-expressing) SPN identification was accomplished using antidromic spikes elicited by stimulating the substantia nigra (Ryan et al, 1989); because this technique likely produces false negatives (Mallet et al, 2006) and because irresolvable cells responding to stimulation may confound interpretation, commentary concerning the subset of identified cells is restricted to those that responded unambiguously (better isolated than most and with minimal overlap from co-responding units) to nigral stimulation and is not necessarily representative of the entire population of striatonigral SPNs. Serendipitously, we also found evidence of antidromic spikes in fibers of passage (detailed in the second portion of this study).

A parallel bipolar electrode having uninsulated tips 0.10 mm long and spaced 0.25 mm (WEST30.1A10; Microprobes, Gaithersburg, MD) was included in some microdrives for implantation into the SNr, at 3.25 mm. posterior to bregma, 1.28 and 1.53 mm lateral to midline, and 4.25 mm below dura. Stimulation of all nearby excitable neuronal elements using current intensities varying between 100-500 uA elicited antidromic spikes. Stimulation patterns consisted of either x number of biphasic pulses 0.5 msec in total duration having varying interstimulus intervals as noted, or of x number of monophasic pulses of 0.05 msec total duration having varying interstimulus intervals, as noted; trains were applied every 20 seconds using a stimulus isolator (A-365; WPI, Sarasota, FL) triggered by a Master 8 (A.M.P.I., Jerusalem, Israel). These responses were recorded in the striatum and identified as either potential direct pathway SPNs (based on SPN metrics and saved for the first portion of the study) or possible fibers of passage (and saved for the latter portion of the study) if the antidromic response fit the following criteria: lower response latency (putative fibers <2 msec., SPNs ~6-13 msec.) time-locked to the stimulation, and an all-or-none quality at peri-threshold stimulation currents. For all SPNs and three of the four fiber-like units of interest reported responding, further evidence was collected: an absolute refractory period <2 msec, and in collisions with spontaneous orthodromic spikes. A schematic and exemplary responses to stimuli are

Figure 9. Raw Data and Isolated Units. Upper panel – raw data (vertical sub-panels represent electrodes 1-4 from top to bottom) Lower left panels – waveforms from a putative fiber (middle) and SPN (bottom) that responded to stimulation with antidromic spikes. Lower right panels – ISI histograms from respective units to the left. Narrow waveform (full-width at half-max <0.2 msec) and many fewer pause durations >1 sec would normally preclude this neuron from being considered projection type, (i.e. SPN, the only units that project outside the striatum), and most would consider its mean rate (~5 Hz) to be fast enough firing to qualify it as a FSI. The lower unit is putatively identified as SPN due to the wavelshape (>0.2 msec, much longer than putative FFNs) and the log-ISI histogram having an approximately bimodal ISI distribution with several pauses longer than one second and as long as hundreds of seconds (as distinct from TANs, which otherwise share waveform length and but have a reliably leptokurtic unimodal log ISI distribution).

Figure 10. Exemplary Dorsal WT Data under l-DOPA.

Upper right: lesion in non-/dorsal striatum. Upper left: raw data from a recording in this locale. Middle right: average ($\pm$ std) of one isolated unit's waveform from the recording.. Middle left: spike rate of this unit over experiment time (1 sec. bins). Lower right: autocorrelogram of this unit's spike activity (5 msec bins). Lower left: interspike interval (ISI) histogram of this unit's spike activity (log-plot, 10 non-linear bins per decade)

Figure 11. Exemplary Non-Dorsal WT Data under L-DOPA.

Upper right: lesion in non-/dorsal striatum. Upper left: raw data from a recording in this locale. Middle right: average ($\pm$ std) of one isolated unit's waveform from the recording. Middle left: spike rate of this unit over experiment time (1 sec. bins). Lower right: autocorrelogram of this unit's spike activity (5 msec bins). Lower left: interspike interval (ISI) histogram of this unit's spike activity (log-plot, 10 non-linear bins per decade)

Figure 12. Exemplary Non-Dorsal *Pitx3*^{Null} Data under l-DOPA.

Upper right: lesion in non-/dorsal striatum. Upper left: raw data from a recording in this locale. Middle right: average (+/- std) of one isolated unit's waveform from the recording. Middle left: spike rate of this unit over experiment time (1 sec. bins). Lower right: autocorrelogram of this unit's spike activity (5 msec bins). Lower left: interspike interval (ISI) histogram of this unit's spike activity (log-plot, 10 non-linear bins per decade)

Figure 13. Exemplary Dorsal *Pitx3*^{Null} Data under D1 Agonist.

Upper right: lesion in non-/dorsal striatum. Upper left: raw data from a recording in this locale. Middle right: average (+/- std) of one isolated unit's waveform from the recording. Middle left: spike rate of this unit over experiment time (1 sec. bins). Lower right: autocorrelogram of this unit's spike activity (5 msec bins). Lower left: interspike interval (ISI) histogram of this unit's spike activity (log-plot, 10 non-linear bins per decade)

shown in **Figure 14** and throughout the results. Reduction in the variability of response latency shortly following (less than hundreds of msec) any spikes (previous antidromic spikes in this case) may also be seen in some cases (Swadlow et al, 1978; Ryan et al, 1989). SPNs were considered unidentified if any criterion was not met; exemplary collisions are shown in **Figure 15**. Corecorded fiber-like units having similar characteristics but not responding to stimuli are also presented as unidentified non-SPN units.

A Note on Technical Differences Between the Two Studies

For the latter study on putative fibers of passage two mice were used, both male and aged 4-6 months: one C57bl/6 and one Pitx3Null. On each animal a microdrive array was implanted aiming octrodes at the dorsolateral striatum (AP +.4, ML 2.0 - 2.5, DV 1.6 - 2.0 mm. below dura). All other parameters were the same as the first study minus the dosing of drugs and subsequent comparisons.

Statistical Considerations

Each SPN's baseline was calculated as the mean number of spikes per second by counting all spikes recorded during the pre-drug period (defined from the saline injection +35 seconds through the pre-drug-injection period until 35 seconds before drug injection) and dividing by the duration of said period. Each SPN's response polarity was calculated as the sign of the response intensity before taking it's absolute value (thus, a negative indicated a decrease in mean spiking and a positive indicated increase.) Each SPN's response intensity to a drug injection was calculated as the absolute value of the difference of the base10 logarithm of pre-injection (i.e. baseline) from post-injection (i.e., response) mean rates, i.e. $\log_{10}(\text{post mean rate}) - \log_{10}(\text{premean rate}) = \log_{10}(\text{post mean rate} / \text{premean rate})$. The threshold depth for considering a unit as dorsal or non-dorsal was 2.25 mm below dura.

Various metrics including mean baseline rate, response polarity (i.e., increasing or decreasing mean rate), and log (post/pre mean rate) from every recorded SPN were calculated in and exported from Matlab (The Mathworks, Inc, Natick, MA) to an *.xls file. SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA), running (as with all other software) under Windows 7 Enterprise, was used to import this *.xls file and to perform subsequent statistical modeling.

Given a potential (and later verified –data not shown) correlation among SPN metrics within animal, a mixed modeling approach was taken using the GLIMMIX procedure in SAS. Thus, mean log-transformed baseline rates (taking each animal's largest sample of neurons between one or the other dataset, l-DOPA or D1 agonist), response polarity (per dataset) and $\log_{10}$ mean rate change (per dataset) were modeled separately from each other. Model building started with the full 3-way interaction (i.e.,

Figure 14. Antidromic Spike Technical Theory.

Left panels from top to bottom – Mouse parasagittal brain sections and overlaid traces of major structures show that because cortical axons pass through the cerebral peduncle (cp) and terminate after the SNr, their axons may transmit an antidromic spike (2) past a striatal recording electrode (1) to the cortical soma during SNr (and thus potentially cp) stimulation; this response would be expected to happen faster than in SPNs due to saltatory conduction and larger axon diameter, but pass to the SNr directly. Striatopallidal neurons would not be expected to respond to stimulation at all due to their field of projection stopping short of the SNr. Upper and Middle right panels – Coronal mouse brain sections highlight representative SNr stimulation (1) and striatal recording (2) sites. Lower right panel – Representative short-latency (-3.5 - +11.5 msec) responses to nigral stimulation (note representative all-or-none quality of earliest response expected from antidromic spikes at peri-threshold stimulation current).

Figure 15. Representative Antidromic Identification Criteria and Collisions.

Upper left - Nigral stimuli 2.0 msec. apart elicit two corresponding spike responses at the recorded SPN soma Lower left - Same using 0.5 msec interstimulus interval in order to show single response Upper right - Collisions from the same unit's antidromic spikes with its spontaneous orthodromic spikes are evident in the first first and third stimuli and not in those remaining during which no spontaneous orthodromic spikes occurred (note consistent latency of antidromic responses) Lower right – The mean waveform +/- standard deviation of the responding SPN is shown.

baseline*genotype*depth + all 2-way interactions and main effects), which if insignificant was followed by the model having the 3-way without the insignificant 2-way interactions. If the 3-way interaction was not significant, then all two way interactions were included and modeling proceeded by removing highest order most insignificant interactions before moving to the final modeling phase. Since responses from most animals came from only one depth out of two possible, then there was little overlap within animal over depth coverage, i.e., only 3 of 19 animals yielded responses from both depths. Thus, degrees of freedom for F-tests and post-hoc analyses were calculated using SAS's updated Kenward-Roger's adjustment called "kr2" in the "ddfm" option of the "model" statement. Baselines, l-DOPA response polarities (as a binary response) and intensities (as a continuous response), and D1 agonist response polarities (as a binary response) and intensities (as a continuous response) were modeled separately using the above approach.

Only three post-hoc comparisons were planned: the effect of a unit change in baseline rate on dorsal *Pitx3*Null drug responses, the effect of genotype on dorsal responses (i.e. *Pitx3*Null dorsal v. WT dorsal) and the effect of depth within *Pitx3*Null animals (i.e. *Pitx3*Null dorsal v. *Pitx3*Null non-dorsal responses). Post-hoc comparison p-values for differences between specific subgroups of the interactions were corrected with a stepdown Sidak adjustment (a.k.a. Holm-Sidak). We report adjusted, unadjusted and Type III p-values as noted below.

In order to mitigate complications involved in reporting the model results, the reader may find it useful to recall the reference and non-reference groups categorized for each factor. "WT" and "non-dorsal" were taken as reference classes; however, for baseline spiking (which is a continuous variable), one $\log_{10}$ unit higher baseline value is taken as the reference value in order to showcase enhancements in the responses that are due to low baselines since correlations with baseline were negative. This allows comparisons of the average non-reference SPN as the group with enhanced response having a baseline one tenth of the average reference (i.e. a $\log_{10}$ unit higher) (or yielding a positive odds ratio –O.R.– by being the numerator). This becomes especially useful when interpreting O.R.s for interactions, for which the factor precedes the ratio; e.g., "baseline O.R. = 1.23*" defines an Odds Ratio in which the Odds estimate for the average SPN having a baseline rate one tenth that of the reference group (i.e. non-reference category making up the numerator) is 1.23 times higher than the Odds estimate for the reference group, which is the average SPN having a baseline ten times the non-reference (i.e. reference category making up the denominator). (Odds represent the probability of an increase over that of a decrease and are only explicit in the intercept, i.e., are implicit or factor\subgroup-dependent in O.R.s.) Appending a certain subgroup to the O.R. name (e.g., *Pitx3*Null depth O.R.) specifies to which subgroup an analysis was restricted.

CHAPTER 4. RESULTS

Results for DA Agonism in DA-Deficient SPNs

Databases are tabulated below (**Tables 1** and **2**) for the three experiments: baseline comparisons, responses under l-DOPA and responses under SKF 81297.

Basal SPN mean firing in WT and DA-deficient *Pitx3*Null mice

In order to ascertain an effect of *Pitx3*Null genotype on baseline SPN spiking, baseline rates were compared between genotypes and depths. Since the distribution of baseline rates spanned >3 orders of magnitude (0.00097 – 5.94 Hz; median 0.185 Hz, Table 3), $\log_{10}(\text{baseline})$ was used. No significant baseline rate differences between factors were found for this dataset. (**Figure 16**) Given no spiking distinction for DA-deficient SPNs overall, we explored their responses to DA agonism using l-DOPA and D1 agonist.

Response polarity differences among WT and DA-deficient *Pitx3*Null mice

With respect to the canonical rate model, an overall spiking increase or decrease in response to DA agonists has been used as a metric for subtype identification of SPNs (e.g. Liang et al., 2008, Singh et al., 2016). Having noticed both response polarities after the putatively selective D1-type receptor agonist SKF 81297 (**Figure 17**), we explored both datasets separately for differences in response polarity ratios, i.e. odds of increases compared to decreases dependent on possibly full interactions of our 3 factors of interest: $\log_{10}(\text{mean baseline rate})$, depth and genotype.

The baselines dataset includes a total of 198 SPNs from 19 animals split into four groups determined by genotype and depth interaction, i.e. WT non-dorsal and dorsal as well as *Pitx3*Null non-dorsal and dorsal. Seven animals contribute to the dorsal datasets for each genotype (57 SPNs from *Pitx3*Null animals and 46 from WT), whereas non-dorsal data comes from 3 *Pitx3*Null and 6 WT animals (67 and 27 SPNs, respectively). During experiments, we observed firing rate changes that were not immediately obvious as to whether or not cells increased or decreased activity (**Figures 18-21**), especially since SPNs can go many minutes without spiking. Thus we assessed the polarity of any change in firing as an increase, –i.e. positive– if the result of subtracting the $\log_{10}(\text{mean baseline})$ from the $\log_{10}(\text{mean response})$ was positive, and negative otherwise. No allowance was made for SPNs having “no change” in activity for two main reasons; this would require testing increases in rate separately from decreases (*or* removing insignificantly changing SPNs from the dataset, requiring post-hoc classification that nullifies the generality of the model); and there is no particularly well-suited statistical

Table 1. Datasets Used.

	<i>Pitx3</i> Null SPNs	WT SPNs
Baselines (from subset of non-overlapping drug recordings)		
dorsal	57 cells in 7 animals	46 cells in 7 animals
non-dorsal	67 cells in 3 animals	27 cells in 6 animals
I-DOPA		
dorsal	48 cells in 7 animals	37 cells in 6 animals
non-dorsal	55 cells in 3 animals	25 cells in 4 animals
SKF 81297		
dorsal	32 cells in 4 animals	33 cells in 6 animals
non-dorsal	59 cells in 2 animals	29 cells in 7 animals

Table 2. Antidromically Identified Cell Subset.

	<i>Pitx3</i> Null SPNs (antidromically identified)	WT SPNs (antidromically identified)
Baselines (from subset of non-overlapping drug recordings)		
dorsal	6 cells in 3 animals	6 cells in 3 animals
non-dorsal	(N/A)	(N/A)
I-DOPA		
dorsal	6 cells in 3 animals	5 cells in 2 animals
non-dorsal	(N/A)	2 cells in 1 animal
SKF 81297		
dorsal	3 cells in 1 animals	8 cells in 3 animals
non-dorsal	(N/A)	2 cells in 1 animal

Table 3. Baselines between Genotype and Depth.

Genotype	Depth	N Obs	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Pitx	dorsal	57	0.00100	0.04337	0.43982	0.52062	4.79883
Pitx	non-dorsal	67	0.00336	0.08496	0.16785	0.67718	5.21594
WT	dorsal	46	0.00097	0.07507	0.28397	0.81032	5.94051
WT	non-dorsal	27	0.01291	0.03672	0.12216	0.28427	3.66251

Figure 16. Modelled Mean Baselines +/- Standard Errors for Each Group.
Linear y-scale reflects Log10(baselineModeled means +/-respective standard errors for each group.

Figure 17. Unexpected Responses to Selective D1 Agonism.

Upper panels – Exemplary dorsal *Pitx3*Null data under D1 agonism. Upper right sub-panel: lesion in dorsal striatum. Upper left sub-panel: raw data from a recording in this locale. Middle right sub-panel: average (+/- std) of one isolated unit's waveform from the recording. Middle left sub-panel: spike rate of this unit over experiment time (1 sec. bins). Lower right sub-panel: autocorrelogram of this unit's spike activity (5 msec bins). Lower left sub-panel: interspike interval (ISI) histogram of this unit's spike activity (log-plot, 10 bins per decade). Lower panels – same as above but for WT and non-dorsal.

Figure 18. Exemplary Dorsal *Pitx3*Null Responses to I-DOPA.

Upper right – Injections shown using each cell’s maximal duration and rate for x and y-axes, respectively. Lower left – Same injections shown uniformly at 1800 sec by clipping recording starts and ends; “B” here is upper left of upper right panel; panels are read across before down.)

Figure 19. Exemplary Non-Dorsal Pitx3Null Responses to l-DOPA.

Upper right – Injections shown using each cell’s maximal duration and rate for x and y-axes, respectively. Lower left – Same injections shown uniformly at 1800 sec by clipping recording starts and ends; “B” here is upper left of upper right panel; panels are read across before down.)

Figure 20. Exemplary Dorsal WT Responses to D1 Ag.

Upper right – Injections shown using each cell’s maximal duration and rate for x and y-axes, respectively. Lower left – Same injections shown uniformly at 1800 sec by clipping recording starts and ends; “B” here is upper left of upper right panel; panels are read across before down.)

Figure 21. Exemplary Non-Dorsal WT Responses to I-DOPA.

Upper right – Injections shown using each cell’s maximal duration and rate for x and y-axes, respectively. Lower left – Same injections shown uniformly at 1800 sec by clipping recording starts and ends; “B” here is upper left of upper right panel; panels are read across before down.)

test for such sporadic spiking as SPNs exhibit. The usual t-test for firing rate differences presumes a normal distribution of spikes per time binned in x second increments, the standard deviation of which is vulnerable to bias by varying the duration of each time bin, x. Exploration of the effect of varying time bin duration left us with no obvious way to decide on an appropriate increment; thus a t-test procedure on its face did not seem to match SPN spiking patterns and lends itself to bias for which we could not account in order to resolve statistical from biological significance.

l-DOPA response polarities

Modeling the odds of a SPN increasing rate in response to l-DOPA thus revealed a significant effect of baseline rate, which interacted significantly with depth (**Table 4**), but there was no significant effect of genotype. According to model estimates, upon injection of l-DOPA, the odds of a positive response polarity were significantly lower with higher baselines, i.e., there was a significant negative correlation between baseline and the odds of a spiking-increase in response to l-DOPA; specifically, according to the resulting baseline O.R., the average SPN having a tenth the baseline spiking of another had 3.261 times higher odds (Type III $p < 0.0002$) of increasing spiking.

This significant negative correlation was significantly exaggerated in the dorsal striatum compared to non-dorsal regardless of genotype. The baseline O.R. specifically for the average dorsal SPN having a tenth the baseline of another was 7.40:1 (adjusted $p = 0.0010$), while that for non-dorsal was 1.44:1 (untested). These O.R.s were significantly different (depth-baseline O.R. = 5.14*, Type III $p = 0.0082$), i.e., SPNs in dorsal striata had 7.4 times higher odds of increasing spiking, which odds were 5.10 ($7.40/1.44 = 5.10$) times higher than non-dorsal odds of increasing spiking.

D1 agonist response polarities

Upon injection of D1 agonist, the odds of a positive rate response polarity (i.e. a spiking increase) were significantly different in terms of a genotype-depth interaction and a 3-way interaction that included baseline in which (as with l-DOPA) lower baselines predicted a greater chance of an increasing spike rate in response to drug, i.e. a negative correlation with baseline spiking.

Post-hoc comparisons of classes within the genotype-depth interaction (i.e. having set $\log_{10}(\text{baseline})$ to various levels (spanning a half log unit on either side of the average baseline, $\sim -1.32 - -0.32$) in order to account for differences it made since it was involved in a significant 3-way interaction) revealed somewhat lower odds for *Pitx3*Null dorsal SPNs having baselines higher than average to increase activity than WT dorsal SPNs. The other planned comparison was between depths in *Pitx3*Null mice, which gave $p > 0.3$ for every baseline tested. Although not strictly tested during multiple comparisons, a depth gradient in WT was more evident at higher baselines than average (data not shown).

Table 4. Overall Significant Results and Post-Hoc Comparisons for Both Drug Response Datasets.

	l-DOPA		SKF 81297	
	polarity	intensity	polarity	intensity
Genotype	-0.69x	+0.43*	-0.77	+0.025
Depth	-1.36x	+0.07	+1.16	-0.055
Genotype * Depth	N/A	+0.27	-4.05*	N/A
log ₁₀ (baseline)	-3.3x*	-0.27*	+0.51	-0.35*
log ₁₀ (baseline) * Depth	-7.4x*	N/A	N/A	N/A
3-way interaction	N/A	-0.37*	32x*	0.27*
<i>Planned and unplanned comparisons</i>				
WT depth interaction	N/A	Untested	+24.1x**	N/A
log ₁₀ (baseline) correlation with <i>Pitx3</i> Null dorsal intensities	N/A	-0.23	-4.7x*	-0.51*
Difference in log ₁₀ (baseline) correlation made by genotype	N/A	+0.11	-6.9x	-0.35*
Difference in log ₁₀ (baseline) correlation made by depth	N/A	+0.15	-6.6x*	-0.10

(* = p <0.05 adj.)

(** = p <0.05 unadjusted)

Planned post-hoc testing of estimated differences between 3-way subgroups revealed that *Pitx3*Null dorsal SPNs showed a significant effect of baseline changes (*Pitx3*Null-dorsal-baseline O.R. = 4.69*, adjusted $p < 0.0471$, i.e. a unit decrease in these particular SPNs' baselines predicted significantly higher odds of a rate increase than decrease in response to D1 agonism); furthermore, this baseline effect was significantly exaggerated in the *Pitx3*Null dorsal location compared to non-dorsal SPNs (i.e. *Pitx3*Null-depth-baseline O.R. = 6.56:1, adjusted $p < 0.0464$, i.e. a stronger negative association with baseline dorsally).

Comparing *Pitx3*Null dorsal correlation with baseline to WT dorsal correlation with baseline indicated that genotype did not significantly alter dorsal-baseline O.R.s (genotype-dorsal-baseline O.R. = 6.874, adjusted $p = 0.0825$). However, accounting for the difference made by depth to the negative baseline correlation, then comparing genotypes (i.e. *Pitx3*Null depth effect on baseline correlation normalized to the same in WT) yielded an estimated genotype-depth-baseline O.R. = 32.63* (TYPE III test $p = 0.013$), i.e. the depth effect on the negative correlation with baseline was exaggerated significantly in the *Pitx3*Null DA gradient compared to WT, which has no such DA gradient (**Figure 22**).

Overall then, in terms of the 3-way interaction, the DA gradient in *Pitx3*Null animals was “top-heavy” (i.e. more negative baseline correlation for dorsal SPNs) in stark contrast to the “bottom-heavier” depth gradient expectation set by WT. Although statistical significance was not tested for (let alone reached) in most of these subgroups, this overall pattern of “different differences” made to the baseline correlations varying by depth and genotype is nonetheless significant as indicated by the Type III p for 3-way interaction. Closer inspection of otherwise insignificant patterns in individual group estimates (at representative baselines, i.e. $\pm$ a half log unit around the log mean, ~ -0.8 for both genotypes) suggested the tendency of even high baseline dorsal WT SPNs to increase spiking. This made for a much weaker baseline correlation whereas non-dorsal WT SPNs seemed not to correlate so weakly. Comparing this depth effect in WT (WT-depth-baseline O.R. = 0.201x, unadjusted $p = 0.166$) to that in *Pitx3*Null animals (4.69x) revealed a significant inversion (genotype-depth-baseline O.R. = 32.63x) whereby *Pitx3*Null dorsal SPNs in particular maintained a significantly negative baseline correlation that was significantly stronger than that in non-dorsal, and significantly more so (dorsal stronger than non-dorsal) than WT; thus odds were greater for SPNs having lower rates to increase spiking (and higher for SPNs having higher rates to decrease) in dorsal *Pitx3*Null striatum than expected given the WT change in correlation with depth (and given the canonical rate model).

Response intensity differences among WT and DA-deficient *Pitx3*Null mice

In order to test the hypothesis that the DA denervation in *Pitx3*Null dorsal SPNs causes exaggerated spiking responses to DA agonism relative to dorsal WT and non-dorsal *Pitx3*Null SPNs (having greater DA innervation), we recorded SPN responses to l-DOPA (25mg/kg with 5 mg/kg benserazide) or SKF 81297 (1 mg/kg) injections

Figure 22. Response Polarity vs. Log Baseline.

The probabilities associated with these increasing relative to non-increasing responses were modeled and fitted separately for each class. Dorsal data is lighter than non-dorsal (in between is the mean for both, I.e. genotype mean); WT are cool colors (blue shades), *Pitx3*Null are warm colors (red shades). 1= increase, 0=decrease.

following baseline recordings. SPNs responded with varied intensities: from low (< 0.01 Hz) baseline rates having high rate responses (~ 1 Hz) to vice versa and all combinations thereof. These responses were clear on both drugs in both depths from *Pitx3*Null animals, although generally appeared to be less intense in WT (**Figures 18-21**).

The absolute magnitude of the response intensity was taken so as to avoid using the overall mean of responses flanking (and thus tending toward) zero; also subsetting the responses into in-/decreasing would risk the generality of the model via post-hoc classification. Again, because the distribution of differences between responses and baselines spanned a similar range as the baselines (range = $0.00056 - 8.3$ Hz, i.e. ~ 4 orders of magnitude, **Figure 23**; data shown explicitly only for D1 agonist, but l-DOPA responses were similar), the difference between $\log_{10}(\text{response})$ and $\log_{10}(\text{baseline})$ –to which we refer as the intensity– was used for the dependent variable in the model. We modelled the intensity as a function of our three factors of interest: $\log_{10}(\text{baseline})$, depth, and genotype, plus interactions.

l-DOPA response intensities

Upon injection of l-DOPA, both response polarities –i.e. absolute values of both increasing and decreasing $\log_{10}(\text{post/pre rate})$ – were more intense: $0.426 \log_{10}$ units according to modelled mean difference at $\log_{10}(\text{baseline}) = 0$ (Type III $p = 0.0354$) in *Pitx3*Null animals averaged across depth (a linear factor of about 2.7x more intense). They were also more intense the lower the baseline: ($0.269 \log_{10}$ units per unit decrease in $\log_{10}(\text{baseline})$) averaged across all classes. In linear units this is a factor of about 1.9x more intense.

Planned post-hoc tests for the significant 3-way interaction indicated that the slope of baseline in *Pitx3*Null dorsal striatum was not significantly non-zero after Holm-Sidak correction (0.233 , adjusted $p = 0.0635$), was not significantly stronger than WT dorsal slope due to baseline (difference = -0.101 , adjusted $p \sim 0.5$), and was not significantly stronger than *Pitx3*Null non-dorsal striatal SPNs (-0.155 , adjusted $p \sim 0.5$). Exploring the remaining subgroups revealed that the slope of baseline in WT dorsal striata was likely non-zero ($0.335 \log$ units per log unit decrease in baseline, unadjusted $p < .0001$; this corresponds to a linear factor of about 2.2x more intense at a baseline 10% that of reference) and possibly higher than non-dorsal WT SPNs ($0.213 \log$ units, 1.63x linear factor unadjusted $p < 0.0382$). However, the difference made by depth to the baseline's effect (on response intensity) in *Pitx3*Null animals normalized to the same in WT was weaker within the *Pitx3*Null DA gradient: an estimated $-0.368 \log_{10}$ units per $\log_{10}$ unit baseline decrease (TYPE III test $p = 0.0477$), i.e. $\sim 43\%$ that of WT.

These 3-way interaction results overall indicate an inversion of a slight depth effect in WT on the negative correlation of baseline with intensity; whereas *Pitx3*Null dorsal SPN baseline correlations compared to those of non-dorsal *Pitx3*Null SPNs were

not significantly different, this difference was nonetheless significant (0.368 log₁₀ units, a linear factor of ~0.43x) less than WT, where dorsal baseline correlations were likely the most significant and the depth effect on these correlations may or may not have been.

D1 agonist response intensities

Upon injection of D1 agonist, both response polarities (i.e. absolute values of both increases and decreases in spiking) were significantly different in a 3-way interaction of baseline rate, genotype and depth. Direct post-hoc pairwise difference estimates revealed that the significant negative correlation of baseline rate with response intensity (0.345 log₁₀ units of intensity per log₁₀ unit decrease in baseline averaged across all 4 classes, $p < 0.0001$, corresponding to ~2.2-fold increase at 10% baseline) was significantly stronger in *Pitx3*Null dorsal striatum (0.507, adjusted $p < 0.0001$, a linear factor of ~3.2x stronger per 10% baseline) than WT dorsal (0.153): difference = 0.354 log units, adjusted $p = 0.0083$, a linear factor of 2.26x in *Pitx3*Null). *Pitx3*Null dorsal striatum did not differ significantly from *Pitx3*Null non-dorsal striatum (difference = 0.103, adjusted $p \sim 0.4$). Although the depth effect on baseline correlations was inverted between genotypes, this effect was not significant; the Type III p-value represents the difference between genotype dorsal baseline correlations, i.e., a significant difference between at least two of the three subgroups (having already accounted for the doubly referential group, WT non-dorsal.)

Antidromically identified SPNs are deficient in baseline spiking and respond strongly to DA agonism

Having been unable to use D1 agonism to differentiate satisfactorily between striatonigral and striatopallidal SPNs, and in order to potentially uncover a role of D1-SPNs for the response intensity, they were putatively identified electrophysiologically in the hopes of finding them among those increasing activity (consistent with the canonical rate model) and to look for any evidence that their activity opposed expectations. Striatonigral (D1 receptor-expressing) SPN identification was accomplished using antidromic spikes elicited by substantia nigra stimulation (Ryan et al, 1989).

(Because this technique likely produces false negatives (Mallet et al, 2006) and because irresolvable cells responding to stimulation may confound interpretation, commentary concerning the subset of identified cells is restricted to those that responded unambiguously – i.e., better isolated than most and with minimal overlap from co-responding units– to nigral stimulation and is not necessarily representative of the entire population of striatonigral SPNs.) Antidromically identified SPN baselines (all dorsal) include a total of 6 SPNs from 3 *Pitx3*Null animals and 8 SPNs from 3 WT animals. Non-parametric statistics for these data are shown in **Table 5**. The antidromically identified

Table 5. Antidromically Identified Cell Baselines Between Genotype and Depth.

Genotype	Depth	N Obs	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Pitx3Null	dorsal	6	0.01976	0.02027	0.02640	0.08764	0.08764
WT	dorsal	8	0.01183	0.20093	0.31922	0.46390	5.94051

cell baselines were found to be significantly lower (on average -0.937 log units $\pm$ std err 0.297 , $p < 0.05$) in *Pitx3*Null animals than WT. In linear units this corresponds to *Pitx* ($10^{-1.486 \pm 0.111} = 0.0327$ Hz $\pm 0.0095/-0.0074$) and WT ($10^{-0.549 \pm 0.276} = 0.282$ Hz $\pm 0.251/-0.132$) yielding a log difference (aka linear factor) of between 5.83-22.9% of WT centered on 11.6% (i.e. $10^{-0.937 \pm 0.297}$).

Furthermore, no identified *Pitx3*Null cells decreased activity in response to either drug; this made odds infinite with respect to WT decreases and thus precluded polarity analyses. **Figure 24.**

Finally, the response intensities were all higher for dorsal *Pitx3*Null cells than WT. However, given the small sample size in the antidromically identified subset, t-tests were used in order to avoid overcomplicating the models for both drugs with considerations known to be important in previous models (e.g. intra-animal correlation of responses); thus assuming SPN independence allows us to show a preliminarily significant difference between genotypes in both datasets best seen in **Figure 25** as all dorsal *Pitx3*Null striatonigral cells had higher firing rate response intensities than WT striatonigral cells.

Results for Fibers of Passage Mistaken as FSIs

During in vivo electrophysiology experiments for a different project (Sagot and Zhou, submitted), antidromic identification of direct pathway SPNs yielded occasional responses at much shorter latency than expected as seen in **Figure 14** (Ryan et al 1986, and 1989) and of much shorter waveform duration than expected for SPNs (Mallet, 2005); compare units in **Figure 14**.

Four such units were collected and their identification metrics and initial responses are presented by row in **Figure 26**; they demonstrated all-or-none –as opposed to graded-- responses to increasing current intensity and consistently followed the stimulus at precise latencies $0.5 - 1.63$ ms (implying conduction velocities from ~ 2.7 -10 m/sec). For three of the four units in question, responses to further stimulation patterns were collected in order to confirm a refractory period < 2.0 msec during which the second of two stimuli would not elicit a response. (**Figure 26**, two right-most columns) (The second unit from top was not ignored but accidentally discovered while searching in an otherwise uneventful recording, during which stimulations were abandoned due to the lack of SPN responses.)

Altogether the pattern of responses for these units suggested an antidromic nature, so their recordings were scrutinized for evidence of random collisions, collisions being the gold standard identifier of antidromic spikes. Notwithstanding hardware shortcomings for trigger latencies (the analog Omniplex system), we occasionally stimulated soon

Figure 24. Baseline Data (Least-Squared Means) for Putative D1 Cells.

Top panel: modeled means $\pm$ respective standard errors for each group bottom panel: diffogram showing how far the difference between genotype baselines is from equal ($\pm$ standard error). catGT = genotype, logbase = $\log_{10}(\text{baseline})$.

Figure

25. *Pitx3*Null Striatonigral Cells All Hyper-Responsive to Drug Compared to WT.

Response intensity (not absolute value) vs. depth below dura shows the distinctions between genotypes for striatonigral SPN responses to both drugs: I-DOPA left, D1 agonist right. (Note lowest two cells were not included in calculations due to non-dorsal depth.)

Figure 26. All Responding Units Left-most column - average (+/- std) of waveforms isolated from the respective unit's in question. Middle left column - To the immediate right are their respective ISI histograms and rate over time plots. Middle right column – Stimulating (train of two stimuli separated by 100 msec) around threshold current yields a stimulus artifact without the putative antidromic spike (respective to each unit's row) in the upper panels of this column. In the lower panels, the spike is reliably present after increasing the stimulating current. Right most column – Upper sub-panels in this column show (in each respective unit's row) nigral stimuli 2.0 - 2.5 msec. apart eliciting two corresponding spike responses yielding information regarding the putative antidromic spike's ability to follow high frequency stimulation. Setting the interstimulus interval to <1 msec in order to show only single responses to two stimuli (in the lower sub-panels of this column) yields information regarding the putative antidromic spike's refractory period, the presence of which excludes the possibility that the spike response is simply part of a stimulation artifact.

enough after spontaneous spikes to discover such evidence. Collisions are shown in **Figure 27** (and may be compared to those of a neighboring SPN from Fig. 5, upper unit's trace panel). One putative fiber from each animal was occasionally interrupted by what appeared to be the same waveform on the same channel, i.e. unexpectedly absent within msec's after a similar spike when otherwise present on flanking stimuli, and thus strongly reminiscent of an antidromic collision. (Swadlow et al, 1978)

Given the low density of FFNs in the striatum (Luk and Sadikot, 2001, Saka et al, 2002) and no published knowledge of their tendency to cluster, it is very unexpected to find that more than one may be recorded simultaneously, yet exactly such data has been reported (Berke, 2008), and indeed all four neurons in this report were accompanied by at least one (incompletely recovered) and up to four units (two completely recovered) having similarly brief waveforms. (**Figures 28**) Units lacking waveforms due to incomplete recovery (~20-50% false negatives) gave no rate or ISI metrics; however, all had the characteristic brief waveform.

All units had a full width at half-max of 0.08-0.18 msec (compared to SPNs having 0.15 – 0.35 – **Figures 26 and 27**, left-most columns and **Figure 27** right units, SPNs), latency to response of <2 msec, and one unit from each animal was interrupted multiple times by spontaneous spikes. Mean rates varied between ~5-17 Hz, and the units recorded during quiet rest, and motor activity switched to a bursty mode with long pauses reminiscent of reported FSIs (data not shown). (Berke, 2011)

Figure 27. All Collisions Left unit panels – Topmost panel shows (after establishing the latency to response for this spike in the 1.1 – 1.3 msec range) that this first response is early enough to be considered spontaneous; no response follows, but the next response is appropriately timed. The middle panel shows a spontaneous spike in the second stimulus artifact (note exaggerated downward deflection compared to all others during that bout of stimulation (exemplified by the first stimulus and those in the upper panel); even though flanking stimuli produced responses, this one did not. The lower panel shows that the fourth stimulation (red trace) in a train of four spaced 10 msec apart was preceded (~100 usec) by a spontaneous (orthodromic) spike; even though flanking stimuli produced typical spike responses (first 3 black traces, next train not shown), this one did not. Right unit panels - Stimuli artifacts (on all four electrodes, shown vertically) are shown flanking the stimulus of interest, on right of upper panel and left of middle panel. Multiple stimuli are overlaid and colored in these graphs. Spontaneous spikes from the unit of interest precede the stimuli of interest by 0-1 msec; note completely non-responsive antidromic spikes, putative collisions Lower left panel Traces from 49 stimuli at increased current having 100 msec interstimulus interval are overlaid; note unit most prominent on third electrode responding to all but two stimuli for each of which a spontaneous orthodromic spike apparently precluded the antidromic response; lowest left panel shows close-up of stimuli, responses, and putative collisions of interest for the same data.

Figure 28. All Neighboring Units and Two Co-responding SPNs All units on left (and corresponding identification metrics to their immediate right) were recorded simultaneously with the respective responding units from Figure 27. (Only the second unit from top of Figure 27 is missing a neighbor due to incomplete recovery; in its place is another neighbor from the recording of the 3rd unit from top.) Upper right unit – The mean waveform $\pm$ standard deviation of a responding SPN previously recorded is shown. ISI histogram of the unit showcasing log normal distribution biased towards longer pauses. In the panel just below a collision between the same unit's antidromic spike in response to the first of four stimuli is not evident (left half), theoretically due to the spontaneous spike's lagging the stimulus by enough time to allow it to pass the stimulated portion of axon before an antidromic spike is elicited there; however, the next spontaneous spike (right half) precludes the antidromic response in the first of the latter four stimuli, theoretically due to collision of the two between the point of recording (striatum) and the point of stimulation on the cell's axon (SNr). Lower right unit – The mean waveform $\pm$ standard deviation of a putatively responding, co-recorded SPN is shown to left of ISI histogram showcasing log normal distribution biased towards longer pauses. In the lowest right panel putative SPNs responded at lower thresholds than the unit of interest, as seen most prominently in the first electrode (and less so on the fourth).

CHAPTER 5. DISCUSSIONS

Discussion for DA Agonism in DA-Deficient SPNs

Interpretation within the Canonical Rate Model

In this paper, we demonstrate some long-term effects of DA denervation on SPN spiking responses to acute DA agonism: namely, a more drastic rate change in response to DA agonism among SPN populations having low baseline spiking and/or located in the DA-denervated *Pitx3*Null dorsal striatum. However, this may not be a direct receptor influence on spiking since it was found in both dorsal, DA-denervated, and non-dorsal, having residual DA, *Pitx3*Null striatal regions (see further discussion on depth effect). We interpret these results on the basis of a modified understanding of the canonical rate model allowing for coactivation of striatonigral and striatopallidal cells during behavior (e.g. Sippy et al., 2015, Cui et al., 2013) as well as for network effects that may overwhelm the effects of DA receptor stimulation on spiking (Ruskin et al., 1999, Nambu, 2008, Beeler et al., 2012, Friend & Kravitz, 2014). Given the supersensitized DA receptors in DA denervated *Pitx3*Null dorsal SPNs, we thus expected two primary effects of DA agonism: stronger spike-rate intensities in *Pitx3*Null dorsal than *Pitx3*Null non-dorsal SPNs and than WT dorsal SPNs. Our interest in polarity was exploratory inasmuch as modifications to the canonical rate model muddy the clarity of its polarity predictions.

In testing for significant group differences and effect sizes in our factors of interest, two notable patterns emerged; our predictions were upheld in genotype but not depth differences, and baseline spiking had a significant (usually main) effect across the entire dataset. Including baseline as a covariate was intended to account for possible floor or ceiling effects on rate changes given baseline spiking (e.g. increasing spiking from a high baseline is necessarily less of an effect than doing so from a low baseline). Furthermore, the higher odds of SPNs increasing activity if they have lower baselines combined with the same correlation for intensity implies that increases tended to be more intense. This may have implications for DA's effects on striatal spike coding of behaviour.

Genotype differences

WT mice placed into an open field and given a behaviorally inert drug, l-DOPA (Kim et al., 2000, even after slight DA depletion Costa et al., 2006, although see Huot, 2015 for a comprehensive review), yield spiking responses used as a control for this dataset, reflecting background neuronal processes and/or any changes in open-field behavior-related spike-firing as the session runs its course. WT responses to ~2 fold extracellular [DA] increase (Abercrombie et al., 1990) are expected to provide the control against which presumptively vigorous SPN receptor responses to l-DOPA in *Pitx3*Null yield spiking changes more intense than WT but with a nonetheless similar ratio of

putatively D1-based excitatory and D2-based inhibitory responses. Bearing this in mind, then interpreting the pattern of baseline correlations (at any level or group) with the dependent variables requires knowledge of the “normal”, i.e. WT, dorsal striatal correlation of baseline with the dependent variable. Thus expected differences between subgroups in the baseline correlations become a central theme in discussing complex results.

These expectations change somewhat for D1 agonist, SKF 81297. While the authors know of no awake WT data correlating baseline differences with response differences, WT locomotive activity is known to increase significantly only at doses 0.5-3 times that used here (Xu et al., 1997, Usiello et al., 2000, Kim et al., 2000), although overall changes in SPN spiking are less straightforward. For example, adding SKF-81297 to the iontophoretic co-application of glutamate and a D2 agonist in anesthetized mouse ventral striatum inhibited the glutamate-induced ~4-5Hz baseline firing rate (relatively high considering our dataset range, after log₁₀ transform, of ~ -3 - +1, mean ~ -0.8) (Xu et al., 1997). In another example, strict application of SKF-81297 in a similar setting for dorsal striatum showed no obvious correlation with baseline for polarity or intensity. (Xu et al., 2011) Finally, Delfino et al., 2007, found that motor cortical activity was enhanced in hemi-lesioned rats having their BOLD response measured, suggesting that motor network glutamatergic striatal input plays a larger role in the D1 agonist response than in the l-DOPA response for WT. Thus expectations are that high-rate non-dorsal SPNs in WT may tend to decrease in rate albeit after drug during locomotor increases, significant or not, and any dorsal baseline correlation should be weak, if present at all.

Genotype differences arose at four points in our study; most noticeably in the much lower baseline spiking from antidromically identified cells, but also in the l-DOPA and D1 agonist intensities as well as D1 agonist response polarities. In the l-DOPA dataset this manifested as a main effect of genotype as well as a complicated interaction of genotype depth and baseline. A similar interaction held in the D1 agonist response polarities. Finally, a genotype difference in D1 agonist response intensities was evident in the dorsal subgroups.

Baselines

The baseline difference in antidromically identified SPN spiking between the two genotypes is interpreted as a direct consequence of DA denervation in the *Pitx3*Null animal. The only manipulation in this experimental system is that of lost ventral tier SNc DA neurons (and aphakia, which was shown to allow similar rearing behavior in blinded versus intact WT controls in Kas et al., 2008); thus, the most parsimonious explanation for the baseline spike difference in the brain is the striking DA loss there.

This result is particularly important in light of the baseline behavioral phenotype in *Pitx3*Null mice. These mice have less spontaneous rearing, take longer and more steps to traverse the challenging beam, take longer to orient downward in the pole test (Hwang et al., 2005), take longer to remove adhesive from snout (Fleming et al., 2013). take less

time to fall from the rotarod (Beeler et al., 2009), more time to traverse the balance beam, more time to complete the string test, and shred less cotton than WT (Singh et al., 2007). These are consistent with canonical rate model and the behavioral effects of deficient DA agonism on striatonigral SNr afferents. (Rommelfanger & Wichmann, 2010)

l-DOPA responses

l-DOPA's effect on SPNs' spiking was clearly more intense in *Pitx3*Null than WT mice (0.426 log units = 2.67*higher on average), which validates our prediction that *Pitx3*Null SPNs are hyper-responsive to DA agonism compared to WT. Publications quantifying spike-rate response intensities to l-DOPA in freely-moving WT mice are not known to us, therefore we can only speculate that given their lack of behavioral response, their spiking responses would be expected – at the crudest level – to similarly lack intensity.

The similar extent to which WT dorsal and *Pitx3*Null dorsal SPN baselines were negatively correlated with spiking change intensities to l-DOPA may be expected if the normal WT reduction in motor behavior in the open field over periods longer than minutes (Costa et al., 2006) corresponds to any grossly similar phenomenon reflected in SPN spiking. Since baseline was negatively correlated with intensity for both datasets, this may reflect a normal condition of SPN spiking changes (most strongly suggested by the WT dorsal negative baseline correlation on l-DOPA, unadjusted $p < 0.0001$).

Furthermore, the significant 3-way interaction in the l-DOPA intensity dataset implies a “top-heavy” WT depth differential in terms of baseline correlations that in the *Pitx3*Null animals is significantly less different. Our explanation for such an unexpectedly complicated finding is only speculative. That a lower baseline will tend (whether or not significantly and even under drug-free or low-dose conditions) to have a stronger response and vice versa (higher baseline having less response) may be expected to differ between WT depths (again, whether or not significantly); although any open-field electrophysiological responses to l-DOPA in WT mice are not known by this author in the literature, sensorimotor (i.e. dorsal, West, 1998) SPN spiking intensity reversing over time in the open field in WT as their exploratory behavior subsides might be one way this could happen. This “top-heavy” difference contrasted greatly with the *Pitx3*Null depth difference wherein non-dorsal responses more closely matched dorsal responses,

The weaker depth difference in *Pitx3*Null mice evident in the significant 3-way interaction may indicate a few possibilities. Since both *Pitx3*Null and WT non-dorsal striata have some or full DA innervation, their different differences with respect to dorsal striatum likelier involves some non-DA-ergic effect above and beyond or even instead of supersensitive receptor stimulation; e.g. regardless of the origin of hyper-responsive SPNs (without being able to rule out non-dorsal SPNs as the initiators), the overall effects of DA deficiency on the negative SPN spiking correlation with baseline are reflected throughout the striatum. E.g. it could be the result of altered motor network processes recruiting otherwise quieter non-dorsal striatal regions once a drug effect has begun (as in

striatonigrostriatal loops in primates shown anatomically by Haber et al., 2000, or the split circuit hypothesis advanced by Joel & Weiner, 1994, also see Beeler et al., 2012 for network simulation, and Beeler et al., 2008 for *Pitx3*Null relevant results). Another possibility could be a developmental compensation, as slight as the non-dorsal DA loss, of the non-dorsal baseline correlation, ensuring more responsive SPNs (notwithstanding dorsal *Pitx3*Null motor effects of DA denervation being independent from development as shown in Li et al., 2015). Similarly any non-dorsal compensatory change (developmental or otherwise) may have an overall network effect that reinforces the baseline correlation in individual SPNs (although see Bamford et al., 2004) leading to the overall pattern observed.

D1 agonist, SKF 81297

Similarly parsing the significant 3 way interaction from D1 agonist response polarities, the WT tendency for a dorsoventral gradient of negative baseline correlation with rate increases to l-DOPA was significantly inverted in *Pitx3*Null mice. This may be evidence that lacking DA in the *Pitx3*Null dorsal striatum allows higher baseline SPNs to pathologically decrease activity in response to DA stimulation and/or makes some less direct contribution to reduce the likelihood (albeit possibly insignificantly on its own) of an increase in rate in the non-dorsal region.

Also noteworthy in the D1 agonist polarity results is the genotype-depth interaction independent of baseline: a striking ubiquity of WT dorsal rate increases in response to a selective D1 agonist, SKF-81297. In contrast, *Pitx3*Null SPNs tended to decrease rate (approaching $p = 0.05$) in response to D1 agonism. This is weak evidence for D1 agonism supporting a rate decreasing role in DA-deficient SPNs. (Johansen et al., 1991, Xu et al., 1997, Fienberg, 1998)

This assertion is further supported by the 3-way interaction (i.e. including baseline as a covariate). *Pitx3*Null dorsal SPN response polarities to D1 agonism may be more sensitive to their baseline rates than those of WT SPNs given the ~ 7-fold stronger negative correlation (notwithstanding its own statistical insignificance) where acute D1 agonism reached SPNs in an otherwise DA deficient context. This is supported by the significant *Pitx3*Null dorsal baseline correlation significantly stronger than non-dorsal (discussed below), which pattern was significantly inverted between genotypes (discussed above). Thus a preponderance of evidence supports a role for DA deficiency in dorsal SPN spiking decreasing in response to acute D1 specific agonism in behaving mice as has been shown in other preparations and species (anesthetized Ballion et al., 2009, Tseng et al., 2004, and in vitro Nisenbaum et al., 1992, Johansen et al., 1991), especially if they have low baseline spiking.

A WT control situation for response intensities to D1 agonism is expected to be different from that to l-DOPA. WT animals are known to respond to SKF-81297 with increases in locomotor activity (Xu et al., 1994, Xu et al., 1997, Usiello et al., 2000, Kim et al., 2000), albeit more reliably at slightly higher doses. Simultaneous

electrophysiological responses to systemic D1 (full, i.e. not partial) agonism are not known in the mouse literature, although anesthetized data have shown primarily significant decreases in spiking in ventral striatum using iontophoresis (Johansen et al., 1991, Fienberg et al., 1998, Xu et al., 1997, also see Gonon note accompanying Gonon, 1997 online).

Electrophysiological responses in dorsal WT striatum to D1 agonism in vivo may be expected to decrease if partial D1 agonist SKF38393 may be compared to SKF 81297. Systemic administration of SKF 38393 did not differ from SKF 81297 in terms of rescuing electrophysiological metrics after DA-depletion (Tseng et al., 2004), and it resulted homogeneously in increases (both in- and out-of-phase) having no obvious baseline correlation in WT animals. (Xu et al., 2011) (Results from these last two studies are also noteworthy for their opposing results when D1 agonist was given to DA deficient animals; Tseng et al., 2004 found that SKF-81297 decreased spiking, whereas Xu et al., 2011 found a statistically insignificant increase; thus expectations for *Pitx3*Null dorsal SPNs responses to D1 agonist are less clear.)

Our data showed a genotype difference in baseline correlation with response intensity; stronger specifically in *Pitx3*Null mice. WT animals showed responses for which we made no planned comparisons for checking against literature expectations, although there was an overall baseline correlation for both genotypes, which suggests a perhaps unexpected result given no previous reports. Nonetheless, *Pitx3*Null response intensity correlations with baseline spiking were significantly stronger. If WT data control for D1 receptor stimulation enhancing response intensities through a network effect (due possibly to extra-BG D1 receptor stimulation) that favors low-spiking SPNs, then we may interpret the remainder of the genotype difference in this metric to the contribution of super-sensitized D1 receptors.

A similar explanation for genotype's main effect in the l-DOPA intensity dataset – i.e. behavior-relevant WT spiking – may underlie the lack of such an effect in the D1 agonist response intensity dataset; to the extent that WT mice respond behaviorally to SKF 81297, their motor network activity (including SPN spiking) should change accordingly. If this involves any changes in SPN spiking intensity (increasing *or* decreasing), then as intensities approach those of the *Pitx3*Null animals, slighter genotype differences become more difficult to detect in such highly variable data. This may indicate the need for a more sensitive metric (or alternatively much more data) in order to show a reduced difference, which is compatible with results from Xu et al., 2011: no effect of DA deficiency in the responses to D1 agonist due to an insignificantly higher spiking increase in paraquat/maneb-treated rats compared to controls.

Indeed, given the intense D1 and D2 receptor expression in SPNs (Levey et al., 1993, Yung et al., 1995) and inherently less motor-inducing effect of D2-SPN stimulation during l-DOPA (Li & Zhou, 2013, Bateup et al., 2010, Darmopil et al., 2009), more intense changes in spiking are expected following DA depletion. (Murer & Moratalla, 2011), but without a way to specify which receptors on which cells will be stimulated and in what network context, assumptions are made instead of controlling for these variables:

e.g. D1 receptor stimulation having its most intense effect directly at SPNs, especially DA-deficient SPNs, or contributions of receptor stimulation being distinguishable between genotypes on a background of more motor network activity than simply glutamatergic cortical input. Interpreting a receptor contribution to significant SPN spiking changes above and beyond WT responses as a mechanism underlying DA-deficient striatal network activity during behavior will thus require future studies: these should control for changes in intrinsic, afferent, recurrent, and/or reentrant network activity (ideally minimizing SPN spiking variability) so that any aspect of the hyper-responsive spiking in DA deficient SPNs can be isolated to differences in receptor activity.

Striatonigral cell response intensities

The canonical rate model developed by Albin and DeLong (Albin et al, 1989 Wichmann & DeLong, 1996) predict that DA receptor supersensitivity in the DA denervated context would allow DA agonists to lead to electrophysiological (and ultimately behavioral) changes via differential dopamine receptor activation in in-/direct pathways. Specifically D1 receptor signaling on D1-SPNs (which is coupled to G-alpha-olf cascades) would be activated to an unusually high degree, which would be expected to exaggerate D1-SPN spike firing simultaneously unusually strong D2 receptor signaling (Gi-coupled) would be expected to decrease the spiking of D2-SPNs. Together, these cellular responses would yield an overall increase in motor activity, more so in the parkinsonian condition than the usual condition. However, this simplified explanation superficially conflicts to some extents with studies reporting increases in the activity of both D1- and D2-SPNs during movement (Cui et al, 2013) as well as those reporting spiking decreases in vast majorities of SPN recordings during free movement and local application of specific DA agonists (Inase et al., 1997; Kiyatkin and Rebec, 1997 and 1999) and specifically in antidromically identified SPNs during movement induced by systemic DA agonism (Ryan et al, 1989). This could conceivably be due to task-relevant cortical glutamatergic input as a prerequisite for spiking at all (Mink, 1996, Beeler et al, 2012, Friend & Kravitz, 2014; also see Discussion).

Antidromically identified cell response intensity differences –polarity being precluded from testing given so few decreases in rate– between genotypes, while not significant in a strict statistical sense, appear to follow rate model expectations and our hypothesis that *Pitx3*Null SPNs are hyper-responsive in terms of intensity to both l-DOPA and SKF 81297. However, a major problem with this interpretation emerges when considering the possible significance of baseline correlation with response intensity so evident in the other datasets –not tested in this dataset due to such a strong correlation of baseline with genotype and our desire to minimize model complication; *Pitx3*Null baselines are significantly lower than WT, and their responses to both drugs appear to be exaggerated over WT cells. It is thus conceivable that the negative correlation of baselines in other datasets (for SPNs from both genotypes) holds here, which (given baseline differences between genotypes in this dataset) may be responsible for the response intensity difference between genotypes; i.e. this would make it easy to confuse a

significant effect of baseline on striatonigral response intensities with a significant effect of genotype. Distinguishing this possibility would require more identified cells having higher baselines in *Pitx3*Null animals and more having lower baselines in WT animals. Yet if the current dataset is representative of the expected ratio of such SPNs, then this experiment appears to be prohibitively difficult since, given our technical capabilities, one to two identified cells per month was the expected rate of acquisition; having two low baseline cells per 8 WT alone would thus require ~5 times the investment, in order to acquire an n of even just 4 of these less likely baseline cells (let alone considering animal N or the fact that all *Pitx3*Null striatonigral cells had low baselines), i.e. a single “rare baseline” identified cell per 3-6 months.

Although inconclusive, these response intensity data still suggest that *Pitx3*Null DA-deficient striatonigral cells are likely hyper-responsive to D1-receptor stimulation whether that be due to a baseline difference and/or receptor supersensitivity. As with interpreting other datasets, any motor network contribution to the response is still difficult to parse from the direct effect of D1 receptor stimulation. Thus, overall only behaving animal experiments recording from SPNs having identified baseline rates and certain receptor expression as well as isolating DA receptor stimulation as locally in time and space as possible may elucidate differences between lesioned and intact striata of the in vivo pharmacological effects of DA agonism on SPN spiking. A combination of antidromic identification with optogenetic and/or designer receptors activated exclusively by designer drugs (DREADDs) coupled with iontophoretic agonist drug injection during systemic antagonist drug application may be most suitable for this purpose.

Depth differences

Although depth made a (different) difference to the response polarities in both genotypes, differences in response intensities expected by our hypothesis were notably lacking except in 3-way interactions. Dorsal depth was associated in both genotypes with a more intensely negative correlation of baseline with l-DOPA response polarities, and the same held true for D1 agonist response polarities in at least *Pitx3*Null animals (according to planned comparisons). For response intensities, we expected depth to interact with genotype in order to differentiate SPN rate change intensities only in *Pitx3*Null dorsal from non-dorsal and from all WT; instead it was useful in distinguishing an inverted baseline correlation between genotypes (wherein *Pitx3*Null non-dorsal SPNs' response intensities were unexpectedly higher) for l-DOPA and in determining that *Pitx3*Null dorsal baseline correlations were stronger only than WT dorsal baseline correlations in the D1 agonist dataset.

In the case of l-DOPA, past research indicates that given 1:1 D1:D2-SPNs in the dorsal striatum (Valjent et al., 2009), the original rate model predicts 1:1 increasing:decreasing polarities (and thus no significant group differences) in either genotype, regardless of whether or not they respond to l-DOPA. Thus, the difference between response polarities dependent on baselines is unexpected by the rate model (since no known results quantify a significant difference between SPN subtypes based on

purely electrophysiological properties in awake animals [Friend & Kravitz, 2014], e.g. long-term baseline spiking). This baseline correlation depending additionally on depth is even further unexpected given (again) the lack of results quantifying differences between SPN subtypes in their electrophysiological properties (Friend & Kravitz, 2014). Either the rate model must be modified to account for an as yet unproven correlation between baseline spiking and SPN subtype, or the model must be updated with another factor having primary influence over spike rates (likely thalamic and cortical glutamatergic input, Mink, 1996, Beeler et al., 2012).

Only 4 of 33 dorsal WT SPNs (from 6 animals) decreased rate responding to a specific D1 receptor agonist; this is exceedingly difficult to reconcile with the rate model's predictions without relying on other factors to explain the phenomenon (e.g. the primacy of cortical input, Beeler et al., 2012, Neve, 2010). Thus, overall polarity results in WT imply – aside from an otherwise paradoxical mechanism through which D1 agonist inhibited spiking in so many non-dorsal SPNs – SPN subtype independence from response polarity. The likelihood that not one of these were D2-SPNs is very low (one sample proportion z-test $p < 0.0001$), given the knowledge of the ~1:1 ratio of SPN subtypes occurring in striata from many species (Valjent et al., 2009). Excluding “insignificant” responses (based on comparing mean rates from 10 or 100 second bins pre- and post-drug) in order to distinguish from the rest of the SPNs those having statistically meaningful increases made the proportion indistinguishable from 50:50; however this approach also uncovered a SPN that significantly decreased mean spiking *(data not shown). This result begs the question of what to expect when stimulating specifically D1 receptors in WT, let alone DA deficient, striatum; thus, the utility of this metric for identifying SPN subtype is dubious and the depth difference considerable enough to require inclusion of other factors predicting rate change polarity.

Of particular note considering depth results from all datasets is that *Pitx3*Null animals only showed a significant effect of depth in terms of response polarity to D1 agonist, i.e. despite our expectations, depth did not play any role in terms of response intensity differences regardless of drug without comparison to an exaggerated WT depth differential of baseline correlation (l-DOPA) or restricting baseline correlation comparison to dorsal regions between genotypes (D1 agonist). This is born out in (**Figures 18-21**) where occasional non-dorsal *Pitx3*Null SPNs have responses apparently as intense as the dorsal SPNs. The supersensitized receptor explanation for such responses would be directly controverted unless these errant SPNs had baselines lower than expected for their response; such cells were not rigorously identified, but the few most obvious had baselines not noticeably lower than their dorsal counterparts (also reflected in the overall results). As explained in the genotype differences section (and more below), results involving the 3-way interaction that included depth differences suggest that altered *Pitx3*Null motor network functions specific to depth may underlie the effects of DA restoration in the *Pitx3*Null dorsal striatum compared to WT dorsal (enhancement) and *Pitx3*Null non-dorsal (comparability) of the negative correlation between SPN baseline and response intensity.

Overall, aside from the particular relevance of depth in the case of the *Pitx3*Null DA denervation gradient, these results might fit into the literature in terms of task-dependent functional organization of the striatum, provided baseline correlation with response intensity is a natural electrophysiological phenotype of SPN spiking subject to the influence of DA denervation and restoration, which is evident in our data (unplanned comparison in WT dorsal striatum on l-DOPA). West (1998, also see West et al., 1990) demonstrated more than half (and up to 100%) of rat dorsolateral striatal (encompassing approximately the dorsal and lateral quadrant of the striatum) SPNs firing sensorimotor related spikes. Graybiel lab studies in primarily dorsolateral (approximately the dorsal and lateral striatal quadrant) striatum depend on such sensorimotor relationships and define their SPN subsets in terms of task-relevance (Kubota et al., 2009, Hernandez et al., 2013, Smith & Graybiel, 2013); they also showed (Xu et al., 1997) that the D3 receptor mutant mouse's increased locomotor phenotype is likely due to electrophysiological changes somewhere other than ventral striatum.

Combining insights from both depths, Belin & Everitt, 2011, showed that chemically inactivating dorsal striatum after a ventral striatal lesion in the opposite hemisphere decreased drug-seeking behavior; they interpreted this effect as an interruption of habit expression. In *Pitx3*Null mice specifically, Beeler et al., 2008 showed that the dorsal DA lesion precludes a normal locomotor response to cocaine and subsequent sensitization to repeated exposure but that ventral striatal functions like conditioned place preference and cocaine sensitization of sucrose preference were spared. Altogether then, – and to the extent that our non-dorsal data represent ventral striatal function – if indeed *Pitx3*Null DA denervation prevents the DA-dependent transfer of ventral striatal (goal-directed) SPN spiking to dorsal (stimulus-response habit-related) spiking, then sensorimotor activity in the dorsal *Pitx3*Null striatum on l-DOPA or D1 agonist could be expected to reflect something akin to spontaneous formation of motor habits: provided this phenomenon is enacted preferentially through SPNs having lower baselines – possibly striatonigral SPNs –, then this interpretation matches our results showing comparable activity between *Pitx3*Null depths.

In literature context

l-DOPA, the most common long term dopamine replacement therapy for PD, relieves the brady- and hypokinesia of parkinsonian patients (i.e. increases self-initiated motor activity) and is eventually associated with motor complications referred to as l-DOPA-induced dyskinesias (Olanow, 2009). These motor effects of acute DA agonism are expected to be mediated by DA receptors located where DA's absence has had the opposite effect of slowing and limiting self-initiated movement. “By what striatal electrophysiological mechanisms does l-DOPA have a motor stimulating effect?” is the overarching question that we hope to further answer.

More specifically, what effect does l-DOPA given in the context of DA deficiency have on SPN spiking? SPNs are the largest subpopulation of BG neurons and are uniquely positioned to integrate cortical input and translate that information to output

nuclei (Mink, 1996, Beeler et al. 2012, Friend & Kravitz, 2014); furthermore, they express dopamine receptors heavily (Levey et al 1993; Yung et al 1995), and at least D1-SPNs show supersensitized receptors in parkinsonian humans and rats (Corvol et al, 2002; Tong et al, 2004; reviewed in Herve et al, 2011). These receptors are located in the DA-denervated BG (an important network for motor control, Alexander et al., 1991) of PD and of l-DOPA sensitive animal models, e.g. *Pitx3*Null mice, 6-OHDA-lesioned rodents. Because the absent nigrostriatal neurons would otherwise innervate only the BG to an appreciable extent, DA from l-DOPA is regulated as usual in DA sufficient brain areas. Thus DA deficient BG DA receptors are acutely stimulated upon l-DOPA administration by its neuroactive metabolite, DA, and the subsequent movements must, by definition, be the consequence of a spike output change initiated in any of the subjects' neurons/networks that cause motor activity (in this case the BG motor circuit since all self-initiated animal movement originates in the spiking of such neurons/networks (Nolte, 2002). Taken together, motor activity induced by l-DOPA is often presumed to precipitate from the action of DA on the spiking of cells in the BG network expressing DA receptors in the context of DA deficiency (such a context including supersensitization, plastic changes from DA loss, dysregulated DA trafficking, etc.). But where is the evidence?

In defense of the causal D1-SPN hypothesis

D1 SPNS may be the specific cells at which l-DOPA's effects are necessary for its acute effects on behavior according to the following rationale. The DA that l-DOPA yields stimulates primarily DA receptors, by definition. However, l-DOPA treatment (i.e. usually combined with a peripheral decarboxylase inhibitor, e.g. benserazide or carbidopa) has shown no detectable behavioral effect in DA-sufficient mice (likely due to their sufficient central DA uptake and metabolism still in place, Abercrombie et al, 1990, Kim et al., 2000). ***l-DOPA's motor stimulating effects (both normal and abnormal or dyskinetic) in the chronically DA deficient BG must depend heavily on changes in DA trafficking and/or receptors,*** i.e. likely to be necessary and sufficient (at the receptor scale) to cause what are known as LID (l-DOPA-induced dyskinesia) in patients, and abnormal involuntary movements in rodents.

The possibility that network plasticity in response to DA denervation could preclude all of these effects is evident when one considers the myriad potential hypotheses formed from the wealth of relevant data: altered DA trafficking (e.g. Lee et al, 2008), compensatory mechanisms (e.g. Iravani et al., 2012), effects on output structures and downstream (e.g. thalamo-cortical changes, simulated or otherwise, Rubin et al, 2012, Hauptmann & Tass, 2010), all acting on relatively sparsely elucidated mechanisms in the WT striatal context (e.g. D2 receptors filtering low-activity synapses in Bamford et al., 2004, DA agonists affecting all subsets of neurons in all behavioral contexts in Inase et al, 1997, Isomura et al., 2013). Nonetheless, our data allows refinement of the canonical rate model and challenges to it owing to the relative inattention to striatal electrophysiological effects of DA pharmacology in awake animals.

Based on the evidence reviewed below, it is put forth that D1-SPNs are the likeliest effectors underlying motor activity induced by l-DOPA (i.e. at least a necessary interface between the DA receptors that l-DOPA stimulates and the behavior that it elicits in the context of chronic DA deficiency). Alternative hypotheses concerning l-DOPA-induced behavior may exist; only manipulations at the receptor, cell and network levels conducted in as isolated a manner as possible will validate or disprove their differential outcomes with certainty. However, using our current techniques, this is not possible, thus we rely on literature support for this keystone of our rationale, that D1 receptors expressed on D1-SPNs are the primary causative agents in l-DOPA's behavioral effect in *Pitx3*Null mice. We support this hypothesis by bolstering the weak electrophysiological data for it (and implicitly for its underlying canonical rate model interpretation). (A similar explanation of D1-SPNs causing AIMs may be found in Murer & Moratalla, 2011.)

Because of the lack of known extra-BG (i.e. cortical, thalamic, peripheral, etc.) DA receptors to supersensitize or become subject to dysregulated DA trafficking while their DA inputs remain intact, then DA receptors outside of the BG are exceedingly unlikely to underlie this phenomenon by virtue of their otherwise intact sensitivity and homeostatic DA context. All PD models have DA deficiency well-restricted to some portion of the BG. (However, consider Halje et al's, 2012, finding of cortical dopaminergic interruption of l-DOPA-induced abnormal involuntary movements and less DA terminals in cortex ipsilateral to 6-OHDA lesion as well as Rubin et al's (2012) points about thalamic and cortical DA loss.)

Because l-DOPA metabolism yields DA, a non-specific DA receptor agonist, both supersensitized D2-family and D1-family receptors may be stimulated to cause the observed motor behaviors. However, much evidence that supports D1- over D2-SPNs playing a necessary role comes from Darmopil et al., 2009, Bateup et al., 2010, Li&Zhou, 2013 and others:

- Darmopil et al, 2009, used genetic inactivation of D1 or D2 receptors in 6-OHDA lesioned mice in order to show that D1 (and not D2) receptors played a necessary role in l-DOPA-induced abnormal involuntary movements. It is noteworthy that D1 receptors aren't known to be localized in BG neurons other than D1-SPNs (Kreitzer, 2009; Smith & Villalba, 2008; Rommelfanger & Wichmann, 2010) except for a subset of non-bursting subthalamic nucleus (STN) cells, which (as the rest of the STN) more strongly express D5 receptors (the other receptor in the D1 family) and express D5 receptor mRNA much more strongly than that of all other DA receptors;
- Bateup et al, 2010, showed that selective~70% reduction in D1-SPNs of DARPP-32 (a downstream effector of DA receptors) abolished l-DOPA's motor effect; on the other hand they showed that ~40% DARPP-32 reduction in D2-SPNs had no effect on l-DOPA-induced abnormal behaviors but did reduce behavioral effects dependent on D2-family-receptors (e.g. haloperidol-induced catalepsy);

- Li & Zhou, 2013, found that increases in horizontal and vertical movements *Pitx3*Null to D2-family agonist, ropinirole, contrasted considerably with that of l-DOPA whereas D1-family agonist, SKF-81297, resembled l-DOPA's behavioral response profile much more comparably.

Taken altogether, these studies strongly suggest that D1-SPN spiking is a necessary (and likely sufficient, given the isolation of supersensitized D1 receptors) antecedent at the cell level for acute l-DOPA-induced behavior.

The evidence discussed in favor of this hypothesis thus far is substantial, however single-unit recordings of SPNs during l-DOPA-induced abnormal behaviors should correlate the supposedly causal SPN activity to the resulting behaviors on a comparable time scale, lending it more credence, yet precisely these recordings are extremely sparse in both primates (not one as of Boraud et al., 2002, one thereafter Liang et al., 2008) and rodents (Iversen, 2010, pg. 438; Kish et al, 1999, Chen et al, 2001, Halje et al, 2012.) Anesthetized animals have been studied in much more detail and their SPNs for the most part show responses similar to those in awake animals but having drastically different firing rates than during waking (Kish et al, 1999) and are therefore of questionable fidelity to waking circumstances, especially in the sensorimotor-related dorsolateral striatum (West, 1998.)

*Pitx3*Null mice are an appropriate model of the motor activity induced by l-DOPA for many reasons. Their l-DOPA-induced motor activity (van den Munckoff et al, 2006), which is absent in WT animals (Ding et al, 2007), is responsive to anti-LID therapies, amantadine and bupropion (Francardo and Cenci, 2014), and molecular markers of DA receptor supersensitivity similar to l-DOPA-induced dyskinesia models arise in the *Pitx3*Null striatum subsequent to l-DOPA treatment (Francardo & Cenci, 2014). Together, these indicate interactions between the conceptual levels of behavior and receptors similar to patients and other animal models concerning the l-DOPA sensitivity that arises after DA deficiency. Finally these mice avoid the pitfalls of toxin-lesioned models (e.g. reproducibility of lesion, dependability of response to l-DOPA across animals; Li & Zhou, 2013).

Clearly one problem with using an SPN's change in spiking to characterize receptor expression (and thus projection target as well as putative network contribution) is the ambiguity of responses to DA. Whereas D1-SPNs are expected in the standard model to increase rate in response to stimulation of D1 receptors according to their downstream effectors, and D2-SPNs should show the opposite effect due to D2 receptor stimulation, the ratio of responses seen in this study and others (e.g. Ruskin et al., 1999) imply that further methods are required in order to distinguish D1- from D2- SPNs in vivo, let alone to distinguish the opposite responses that further parse each of those subpopulations since their pharmacological responses could be so similar (e.g. given our results or those of others like Inase et al., 1997, Claussen & Dafny, 2015).

Ryan et al. 1989 tried to disambiguate responses to amphetamine in healthy, mobile rats by antidromically activating putative D1-SPNs (as we have done here for the *Pitx3* mutant mouse), but many of the identified cells responded unexpectedly; they showed general decreases in firing over tens of minutes and specific decreases using behavioral periods matched pre- and post-drug exposure (akin to a “behavioral clamp” experiment, e.g. spiking considered only during head bobbing, only during sniffing, etc.), although some did increase. Rebec, 1998, tried iontophoretic application of DA simultaneously with glutamate into the striatum of unrestrained rats in order to provide more support for the hypothesis that motor-related SPNs were responding to drug (amphetamine in his previous studies), and not to other motor-related brain activity, cortical input or sensory feedback among others (for some of which he performed separate studies to rule out their possibilities).

In awake rats, Kish et al, 1999, observed that SPN spiking increased *on average* after apomorphine (a mixed D1/D2 agonist) in 6-OHDA-lesioned striata whereas apomorphine was without effect on the respective unlesioned striata and unilateral saline control animal striata. They concluded that firing rate sensitivity to DA was increased in DA-deficient SPNs. Chen et al, 2001, saw the same dose of apomorphine, in contrast, decrease rates in lesioned striata *on average* (whose baseline rates were already increased from control). They concluded that DA's function was inhibitory to SPNs and may thereby affect action selection. However, neither study distinguished cells having different rate response polarities; thus, by conflating rate increases with decreases when averaging all rate changes, mechanisms more refined than homogenous rate coding across cells (known to be of different subtypes) were precluded from commentary. Accordingly, Halje et al, 2012, reported that l-DOPA injection after unilateral 6-OHDA lesion indeed leads to both increases and decreases in SPN spiking, but conclusions were focused entirely on an oscillatory cortical activity contributing to dyskinesia. Each of these studies left SPNs unidentified, which weakened their explanatory power, thus limiting clinically relevant interpretations (especially those possible within the canonical framework).

The resulting dichotomy of responses to D1 agonist herein (in-/decreases exemplified best in **Figures 18-21**) was therefore not unexpected (also see other studies classifying SPNs according primarily to task-relevance before or in addition to DA response, e.g. Hernandez et al, 2013, Inase et al., 1997). However, promisingly, SPNs in *Pitx3*Null animals, both increasing and decreasing, responded more intensely accompanying motor activity induced by l-DOPA.

Inasmuch as our conclusions are similar, our results are in agreement with Kish et al. 1999, in the rat 6-OHDA model of LID. We also extend their results in the following ways:

- by the presence of a different style of induced abnormal behavior –“three-paw dyskinesia” in *Pitx3*Null (Ding et al, 2007) instead of contralateral rotation– due to the different DA lesion in *Pitx3*Null mice (Li et al, 2015);

- by antidromically identifying putative D1-SPNs as being particularly and significantly affected by DA depletion and likely by DA agonism in that context.

To this last point, although there is no compelling evidence from awake, behaving animals that specific activation of D1 receptors causes strictly, or even primarily, increases in D1-SPN spiking (as opposed to more complex, modest, or other unexpected effects on SPN spiking), such responses are expected in at least some subpopulation of D1-SPNs secondary to D1 receptor stimulation. This is especially so given concurrent supersensitization of DA receptor signaling after DA depletion, all based on the prediction of the canonical rate model (also supported via parkinsonian primate BG cell responses to DA agonists, Boraud et al, 2002, and many rodent studies, Ruskin et al, 1999, Waszczak, 1984, Iversen, 2010, pg 438) and some in vitro rodent PD model data (Plotkin et al, 2011). From a purely electrophysiological perspective, because optical stimulation of D1-SPNs is known to activate behavior, they would be expected to increase firing based on their being positioned conceptually at the scale between DA-denervated, supersensitized DA receptors and behavior induced by l-DOPA in the putative causal pathway outlined above.

Two WT identified SPNs (not shown since they were below the threshold for being considered dorsal) decreased activity after D1 agonism; these do not fit the canonical rate model but warrant comment since they have been seen before. They may be disfacilitated (Mahon et al, 2006) by a behavior-related, silencing of glutamatergic inputs originating in the cortex and thalamus, discussed more below. Alternatively, in the case of l-DOPA injections D2 receptor agonism may silence these SPNs (Cepeda & Levine, 1998; Plotkin et al, 2011). Although supported in non-waking animal studies and in vitro, these interpretations remain speculative.

Rate-decreasing SPNs identified as D1 expressing (e.g. Fig. 7, Ryan et al, 1989) are difficult to account for because their spike activity in relation to motor behavior is exactly opposite of that expected based on the canonical rate model; thus this subpopulation warrants special attention. Its existence alone is sufficient evidence to warrant reconsidering the model, which has been easily resolved by adding one more level of complexity: that of behaviorally relevant glutamatergic inputs. Behavioral relevance may be built into the topographical nature of the corticostriatal projections: a principled simulation by Beeler et al, 2012, endowed these projections with the ability to facilitate SPN spiking in a task-dependent way. Thus relevant spiking in the cortex was necessary before DA could exert its effect, and the model accounted for DA effects they observed in vivo. In other words, one possible explanation for the motor-relatedness of any single SPN presupposes glutamatergic input sufficient to cause SPN spiking before DA may be allowed to have its canonical rate effect on spiking.

In vitro, other unpublished results from our lab indicate that dorsal *Pitx3*Null D1-SPNs are more sensitive to D1 agonism in terms of excitability and current pulse-induced firing. Observing these results in a preparation lacking much cortical and thalamic glutamatergic input is even more impressive considering that such input has been shown to be necessary for not only SPN spiking (Surmeier et al. 2007) but the D1 agonist effect

as well (Plotkin et al, 2011). Considering this point in conjunction with the findings that glutamatergic inputs are the primary source of glutamatergic excitation to SPNs (Surmeier et al. 2007), then it stands to reason that specific cortical/thalamic activity gives way to specific striatal activity in a topographic manner and that this topography may underlie the specialized functions of specific locales of striatum (Beeler et al, 2012).

Discussion of Striatal Fibers of Passage Being Mistaken for FSIs

Given the width of these waveforms compared to SPNs, and their rates, they would most likely be considered FSIs (PV+ GABAergic interneurons). Even a more detailed look at their patterns during wake and sleep might support that conclusion. However, while the degree of evidence supporting each cell's identity differs, their matching characteristics nonetheless bolster the hypothesis that they are neither interneurons nor SPNs.

The results shown are very unlikely to be orthodromic spikes from the midbrain since collisions would be extremely unexpected by any known mechanism in that case; the consistency of a precise temporal sequence of stimulus then response, the collisions, and the refractory period all point to these responses as axonal spike propagation.

Myelinated cortical axons exhibit conduction velocities on the order of high singles to tens of meters per second in cats (Jinnai & Matsuda, 1979) and macaques (Turner & DeLong, 2000). Sensorimotor cortical axons course more densely in the dorsolateral striatum. (Wilson, 1987, Kubota et al, 2009, Schwarz & Chakrabarti, 2015) Given the only known major projections to striatum that would approach the stimulating electrode placed in this study, these fibers would presumably be of cortical origin. In summary, these characteristics (widespread instances of bundled cell processes projecting through the striatum to or beyond midbrain and having conduction velocities in the single meters per second) increase the candidacy of the cortex as the origin of these fibers.

Conclusions

Overall, we have bolstered heretofore weak electrophysiological data supporting the hypothesis (modeled on the in/direct pathways concept) that hyper-responsive SPNs mediate motor activity initiated via DA-deficient SPN DA receptor signaling by showing clear distinction in SPN spiking changes between dorsal WT and DA-denervated *Pitx3*Null striatum in response to DA agonists. Furthermore, where DA denervation is chronically <1% that of WT (Li&Zhou, 2013), baseline striatonigral spiking is much less than where there is full DA innervation in the complementary WT dorsal striatum. Fitting our results into the context of previously published literature concerning the causes of motor activity induced by l-DOPA, we posit that lowering l-DOPA dose reduces dyskinesias but at the cost of some efficacy in restoring motor activity to the otherwise parkinsonian state. This may imply that l-DOPA's induction of both normal and

dyskinetic motor activity may be inextricably linked to a common pharmacological mechanism involving DA signaling in the BG.

Furthermore, while the given evidence for fibers of passage being mistaken as striatal FSIs does not irrefutably prove the identity/somatic location of the units, it nonetheless casts strong doubt on the validity of the assumption that all striatal units having narrow waveforms and high rate are interneurons. Previous findings may thus be reconsidered, and future reports more cautiously interpreted in order to potentially resolve apparently contradictory results. This may also represent an opportunity to improve the utility of striatal recordings; e.g., upon improving this technique's reliability and identifying these fibers, one may be able to improve the corroboration of striatal data with somewhat coextensive extrastriatal projection cells in real-time without requiring extra electrodes. Future experiments should attempt to distinguish *in vivo* identification criteria between FSIs and fibers of passage through the striatum, most likely cortical.

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VITA

I was born in 1984. After finally deciding on a science degree out of appreciation for the attention to detail and empiricism (the power that is knowledge), I took my time at Cal State Northridge; from 2004-2008 and beyond in the Minehan organic chemistry lab. I developed my fine motor skills, long-lasting and valuable friendships, and very few functional organic compounds. Nevertheless, my heart was set on pharmacology and the understanding of voluntary perception, the responsibility that adults hold for their perspective. Off to UTHSC for a whirlwind of electrophysiology, mouse work, and a hard knock lesson in political mechanics of academic misconduct. It's been quite a ride so far... Here's to another 130 years! (hopefully that's enough time to reclaim my rightful spot as father of gangsta rap guitar covers on video sharing sites.)