CELL-SPECIFIC SPINOPHILIN FUNCTION UNDERLYING STRIATAL MOTOR ADAPTATIONS ASSOCIATED WITH AMPHETAMINE-INDUCED BEHAVIORAL SENSITIZATION

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Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the Program of Medical Neuroscience,
Indiana University

July 2022

Accepted by the Graduate Faculty of Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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DEDICATIONS

I would like to dedicate this dissertation to God, my ancestors, family and friends; mom-I love you, pops-it's never too late, Tiwanda Watkins and Kevin Spivey- you two are the best siblings I could have ever asked for. I thank one of my best-friend's ever, Zachary, for everything you helped me with during this journey. There is no way I could have done this without all of you and for that I will always be eternally grateful. I love y'all always and in all ways! To all those that came before me that had to walk alone so that I could run, I dedicate this work to you as well.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Anthony J. Baucum II, for helping me turn a dream into a reality; I could not have accomplished this goal without you. To the rest of my committee: Drs. Bryan Yamamoto, Andy Hudmon, Brady Atwood, and Marian Logrip; I would like to extend my deepest gratitude for helping me throughout my graduate career. A special thanks to the IUPUI Biology Department for allowing me to refine my teaching skills, which helped me decide and commit to my long-term goal of becoming an academic researcher. In addition, I would like to thank Indiana University School of Medicine Graduate Program and Stark Neurosciences Research Institute for providing this amazing opportunity.

Graduate school can be extremely challenging and there are times in which camaraderie is paramount. There are individuals that made my graduate experience positively unforgettable! I would like to thank Drs. Mike Edler, Braulio Munoz, Brandon Fritz, Staci Engle, and Asma Salek. To my current lab-mates: Cameron Morris, Kaitlyn Stickel and Wesley Corey thank you for all your support and kind words of encouragement when I was really in my own way! In addition, the work in this dissertation are adapted from both first and co-first author works. Specifically, a portion of chapter two was adapted from Edler MC*, Salek AB*, Watkins DS*, Kaur H, Morris CW, Yamamoto BK, Baucum AJ 2nd. (2018). Mechanisms regulating the association of protein phosphatase 1 with spinophilin and neurabin. Nov 21; 9(11): 2701-2712. PMCID: PMC7011719. Also, a portion of chapter three was adapted from A portion of these data were published in Morris CW*, Watkins DS*, Salek AB*, Michael C. Edler, Baucum II, AJ. (2018). The association of spinophilin with disks large-associated protein 3

(SAPAP3) is regulated by metabotropic glutamate receptor (mGluR) 5. Mol Cell Neuro. Jun 14;90:60-69. PMCID:PMC6294707. Lastly, portions of chapter four were adapted from Watkins, DS, True, JD, Mosley, AL, & Baucum 2nd, AJ (2018). Proteomic Analysis of the Spinophilin Interactome in Rodent Striatum Following Psychostimulant Sensitization. Proteomes, 6(4), 53. I would like to thank everyone who helped make the publications possible.

I would like to thank my family. Mom without your undeniable strength I know this would not have been possible! Thank you for teaching me to never give up "…even when things look bleakest there is always a ram in the bush…"! Tisha and Kevin…y'all know what it is! I thank you so much for allowing me to complain for hours about my project and still reminding me that I needed to do this not just for me, but for those that came before me. My heart is overwhelmed by how much it meant for me to hear words of encouragement from you. We did this together in many ways. Thank you!

Finally, as Snoop Dogg once eloquently stated …"I would like to thank myself". There have been multiple moments in which I was ready to throw in the towel and change directions; however, I still stuck with it. I didn't give up and I pursued my goal no matter how slow my walk may have been, or how often I stumbled. This dissertation is a testament to my ability to triumph in the face of adversity. I am humbled and honored to have taken this road. I thank the ancestors and God for holding me through my PhD journey.

Darryl Shumon Watkins

CELL-SPECIFIC SPINOPHILIN FUNCTION UNDERLYING STRIATAL MOTOR ADAPTATIONS ASSOCIATED WITH AMPHETAMINE-INDUCED BEHAVIORAL SENSITIZATION

Striatal-mediated pathological disease-states such as Obsessive-Compulsive Disorder (OCD), Parkinson's Disease (PD), and psychostimulant drug addiction/abuse are coupled with distinct motor movement abnormalities. In addition, these disorders are associated with perturbed synaptic transmission. Proper synaptic transmission is critical for maintaining neuronal communication. Furthermore, in many striatal-dependent disease-states, the principle striatal neurons, medium spiny neurons (MSNs), exhibit differential perturbations in downstream signaling. Signal transduction pathways that are localized to the glutamatergic post-synaptic density (PSD) of GABAergic MSNs regulate protein phosphorylation in a tightly controlled manner. Alterations in the control of this phosphorylation in striatal MSNs are observed in myriad striatal pathological disease-states and can give rise to perturbations in synaptic transmission. While serine/threonine kinases obtain substrate specificity, in part, by phosphorylating specific consensus sites, serine/threonine phosphatases such as protein phosphatase 1 (PP1) are much more promiscuous. To obtain substrate selectivity, PP1 associates with targeting proteins. The major targeting protein for PP1 in the PSD of striatal dendritic spines is spinophilin. Spinophilin not only binds PP1, but also concurrently interacts with myriad synaptic proteins. Interestingly, dopamine depletion, an animal model of PD, modulates spinophilin protein-protein interactions in the striatum. However, spinophilin function on

basal striatal-mediated motor behaviors such as the rotarod or under hyperdopaminergic states such as those observed following psychostimulant-induced behavioral sensitization are less well characterized. To elucidate spinophilin function more specifically, we have generated multiple transgenic animals that allow for cell type-specific loss of spinophilin as well as cell-specific interrogation of spinophilin protein interactions. Here, I report the functional role of spinophilin in regulating striatal mediated motor behaviors and functional changes associated with amphetamine-induced locomotor sensitization. In addition, we define changes in spinophilin protein-protein interactions that may mediate these behavioral changes. Furthermore, global loss of spinophilin abrogates amphetamine-induced sensitization and plays a critical role in striatal motor learning and performance. The data suggest that the striatal spinophilin protein interactome is upregulated in MSNs following psychostimulant administration. In addition, loss of spinophilin changes protein expression in myriad psychostimulant-mediated striatal adaptations. Taken together the data suggests that spinophilin's protein-protein interactions in the striatum are obligate for appropriate striatal mediated motor function.

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LIST OF ABBREVIATIONS

A2A	Adora2a
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANOVA	Analysis of variance
BG	Basal Ganglia
CaMKII	Calcium/calmodulin-dependent kinase II
cAMP	Cyclic adenosine monophosphate
CHAT	Choline Acetyltransferase
CIN	Cholinergic Interneuron
CST	Cortico-Striatal-Thalamic
CNS	Central nervous system
D1R	Dopamine-1 Receptor
D2R	Dopamine-2 Receptor
DA	Dopamine
DARPP	Dopamine- and cAMP- regulated phosphoprotein
dMSNs	Direct Pathway Medium Spiny Neurons
Glu	Glutamate
GluA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid subunit
GluN	N-methyl-D-aspartate receptor subunit
GPCR	G protein-coupled receptor
GPe	Globus Pallidus external segment
GPi	Globus Pallidus internal segment

KEGG	Kyoto Encyclopedia of Gene and Genomes
KO	Knock-out
LTD	Long-term Depression
LTP	Long-term Potentiation
iMSN	Indirect Pathway Medium Spiny Neurons
MSN	Medium spiny neuron
NMDAR	N-methyl-D-Aspartate Receptor
OCD	Obsessive-Compulsive Disorder
PD	Parkinson's Disease
PKA	cAMP-dependent protein kinase A
PP1	Serine/threonine protein phosphatase 1
PP2B	Calcineurin
PSD	Post-synaptic density
SEM	Standard error of the mean
Ser	Serine
SNc	Substantia Nigra pars compacta
SNr	Substantia Nigra pars reticulata
STN	Subthalamic
Thr	Threonine
WT	Wildtype

Chapter I: Introduction

1.1 Basal Ganglia and Motor Function

Movement can be categorized as either voluntary (intentional) or involuntary (unintentional). In all movement generation, myriad brain regions work together to eventually signal to peripheral muscle networks whose concentric and eccentric contractions give rise to coordinated movements. Although the descending motor pathway mediates the ultimate motor action outcome, there are networks within the central nervous system (CNS) that refine, modify, and influence final motor execution (Groenewegen, 2003). Brain regions such as the cerebellum, the cerebral cortex, and the basal ganglia form critical neural networks that give rise to proper motor function. The ability to interact with and respond to changes in our external environment are built upon appropriate voluntary motor actions.

Voluntary movements are achieved when signals from the motor cortex propagate to the brain stem, which in turn project to the spinal cord, eventually transmitting signals that cause the muscles to contract. Brain regions such as the cerebellum and the basal ganglia work to influence and refine the final motor output signals of intentional movements (Bostan & Strick, 2018). Although cerebellum and basal ganglia outputs integrate information from many of the same cortical brain regions, up until recently, they were considered to be completely separate motor networks (Bostan & Strick, 2018). Currently, that the cerebellum and the basal ganglia may indirectly create an integrative functional network between the two structures at the subcortical level (Bostan & Strick, 2018). There is sufficient evidence to suggest that both cerebellar and basal ganglia motor

networks work in concert to achieve complex motor movements; however, the basal ganglia is extremely important in mediating initiation and fine-tuning of voluntary movement planning and motor action.

Skilled voluntary movements involve the synchronized actions from both sensory and motor networks (Dhawale et al., 2021; Schwartz, 2016); for example, when playing a fortissimo accented chord on a piano, the ability to press down on a piano key (motor function) with a certain amount of force (tactile/proprioception) to achieve a particular sound (auditory) which is critical feedback for fine-tuning the motor skill (**Figure 1**). The complex behavior of playing a piano is not only a learned motor action but requires repetition and motor fine-tuning along with proper timing to appropriately execute the skill (**Figure 1**). Execution of complex motor actions like playing the piano or even day-to-day motor actions such as walking to a desired location are precise coordinated signals from CNS motor networks that, over time and with practice, become imprinted and produce motor memory. The basal ganglia play a crucial role in this imprinting and motor memory (Graybiel & Grafton, 2015).

Throughout child development and into adulthood, complex motor movements such as walking, running, swimming, and playing a musical instrument have been refined into skilled motor movements. Skilled motor execution requires that brain regions such as the basal ganglia maintain proper function. Understanding the anatomical and functional connectivity within the basal ganglia aids in the elucidation of, and provides fundamental insight into, the etiology of diseases that cause motor action related dysfunction.

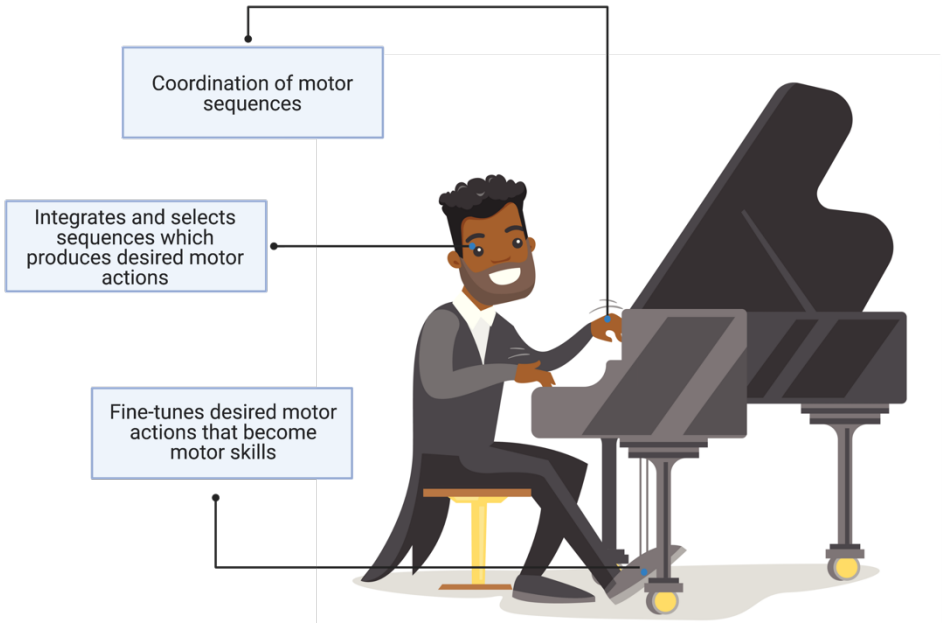

Figure 1: Basal Ganglia Function Mediates Voluntary Movement. The Basal Ganglia is critical for interacting with our environment. Playing an instrument involves the integration of both sensory and motor networks, that require fine-tuning and appropriate timing of motor sequences and actions. The Basal Ganglia is known to mediate these functions. Created with BioRender.com.

1.2 Basal Ganglia Anatomy

The basal ganglia brain regions are subcortical neuronal networks that work in concert with one another to mediate higher order brain function such as executive planning (Lai et al., 2018; Lanciego et al., 2012), the fine-tuning of movement (Aldridge et al., 2004; DeLong et al., 1984; Hauber, 1998; Klaus et al., 2019), motivation (Courtemanche & Cammalleri, 2019; Ikemoto et al., 2015), and reward processing (Doya & Kimura, 2014; Hikosaka et al., 2014; Schultz, 2016). The brain regions within the basal ganglia of humans and rodents consist of the substantia nigra (SN), striatum (rodents) caudate/putamen (humans), subthalamic nucleus (STN), and pallidum (**Figure 2**). Each basal ganglia structure is distinguishably different based on either subnuclei, afferent/efferent projections, or cytochemical characteristics. To better understand the basal ganglia, we will first discuss the anatomical and cytochemical diversity and homogeny within this network per brain region. Understanding where the basal ganglia structures reside within the brain and each structure's different neuronal and biochemical components conveys the true complexity of the network.

1.2.1 Substantia Nigra

The SN is a midbrain structure and considered one brain region; however due to cytological differences and the fact that there are two distinct areas within the SN that send and receive inputs to/from different brain regions, the SN is divided into two subregions: the substantia nigra pars compacta (SNc) and the substantia nigra pars reticulata (SNr) (**Figure 2**). The SNc is mainly comprised of densely packed

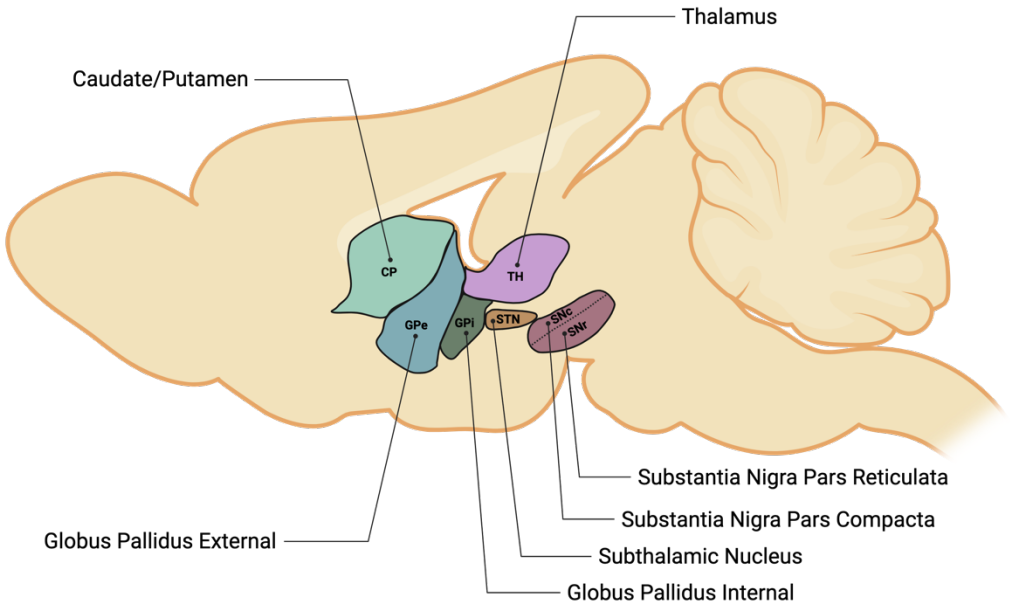

Figure 2: Basal Ganglia Anatomy. Sagittal view of basal ganglia brain regions within the rodent brain. Adopted from mouse.brain-map.org sagittal level [10-12], with lateral thickness of 1.725mm – 2.15mm (Dong, 2008) . Created with BioRender.com.

dopaminergic neurons. L-3,4-dihydroxyphenylalanine (L-DOPA), a precursor to dopamine and neuromelanin, is highly concentrated in the SNc. The neuromelanin causes the cell-bodies in the SNc to appear darker (Fabbri et al., 2017). The SNr consists mainly of tonically active gamma-aminobutyric acid (GABA)-ergic neurons; however, the neurons are structurally very similar to neurons that reside in the internal segment of the dorsal pallidum.

1.2.2 Subthalamic Nucleus

The STN is considered part of the ventral thalamus (**Figure 2**). The STN is located adjacent to the cerebral peduncle (Kita et al., 2014). The STN consists mainly of glutamatergic neurons and GABAergic interneurons that are immunoreactive for calcium binding proteins such as parvalbumin (Emmi et al., 2020).

1.2.3 Globus Pallidum

The dorsal pallidum, also known as the globus pallidus, consists of an external and internal segment, globus pallidus external (GPe) or globus pallidus internal (GPi), respectively, and is located caudally adjacent to the striatum (**Figure 2**). The GPe consists mainly of GABAergic cells that are of two distinct types. The arkypallidal neurons, which account for nearly ~25% of the neurons within the GPe and express preproenkephalin (Abdi et al., 2015), and the prototypic GABAergic interneurons which either contain parvalbumin (~30-40%) or are tonically firing interneurons that also express transcription factors Lhx-6 and Nkx2-2 (~34%) (Abdi et al., 2015; Gittis et al., 2014). The GPi consists of GABAergic neurons that sustain high-frequency discharges

(Nambu, 2007). Much like the GPe and the SNr, GPi neurons are sparsely spined with low dendritic branching (Yelnik et al., 1984).

1.2.4 Striatum

The striatum is the major input hub into the basal ganglia and the initial output hub to other parts of the basal ganglia. The human striatum is comprised of the caudate nucleus, putamen, and nucleus accumbens, with the latter commonly referred to as the ventral striatum in rodents. One major feature in the human striatum is that the caudate and putamen are separated by the white matter brain tracts known as the internal capsule (Báez-Mendoza & Schultz, 2013; Chuhma et al., 2017). In rodents there is no anatomically discernable difference between the caudate and putamen and these two structures are considered the dorsal striatum, although there is some level of division within the dorsal striatum based on behavioral function and innervation. Furthermore, the ventral striatum consists of the nucleus accumbens and the olfactory tubercle in rodents (Chuhma et al., 2017).

Currently, despite minor anatomical differences, the rodent and human basal ganglia's overall functional output are extremely similar. The principal output neurons (~95%) within the striatum are GABAergic medium spiny neurons (MSNs), also sometimes referred to as spiny projection neurons. Interestingly, although very small in number, the striatum has over ten different classes of GABAergic interneurons which make up ~5% of the cells, including neuropeptide Y-expressing, tyrosine hydroxylase-expressing, and calretinin-expressing interneurons (Tepper & Koós, 2016). However, the two major interneuron classes that have been implicated in striatal movement regulation

are parvalbumin-expressing and cholinergic interneurons (Gritton et al., 2019). The anatomical and cytochemical differences within basal ganglia structures suggest specific functional roles that work together to achieve appropriate basal ganglia function. Exploring the functional connectivity of the basal ganglia will provide critical insight into how the basal ganglia influences appropriate and pathological motor behavior.

1.3 Basal Ganglia Connectivity

To achieve proper motor function, it is imperative that the intrinsic connectivity of the basal ganglia is intact. The basal ganglia consist of glutamatergic, GABAergic and dopaminergic projection interconnectivity that allows for precise signaling between the brain structures (**Figure 3**). Multiple regions within the basal ganglia show inversely related activity, in that activation of one region can inhibit another, and vice versa (**Figure 3**). We will focus on the cortico-striatal-thalamic (CST) connectivity and examine the role that the basal ganglia play in motor function. Within the CST, cortical and thalamic excitatory projections integrate in the striatum with dopaminergic inputs arising from the SNc (**Figure 4**) to either increase or decrease activity from cortical inputs that aid in facilitating striatal-mediated motor actions.

The SNr and GPi are innervated by dopamine class-1 receptor (D1R)-containing MSNs from the striatum. D1R-MSNs directly innervate the SNr/GPi (direct pathway)-which innervate the thalamus (**Figure 4**) (Gerfen & Bolam, 2010). Direct pathway D1R-MSNs (dMSNs) release the neurotransmitter GABA (**Figure 3, Figure 4**) onto the tonically active GABAergic inhibitory neurons within the SNr/GPi (**Figure 3**),

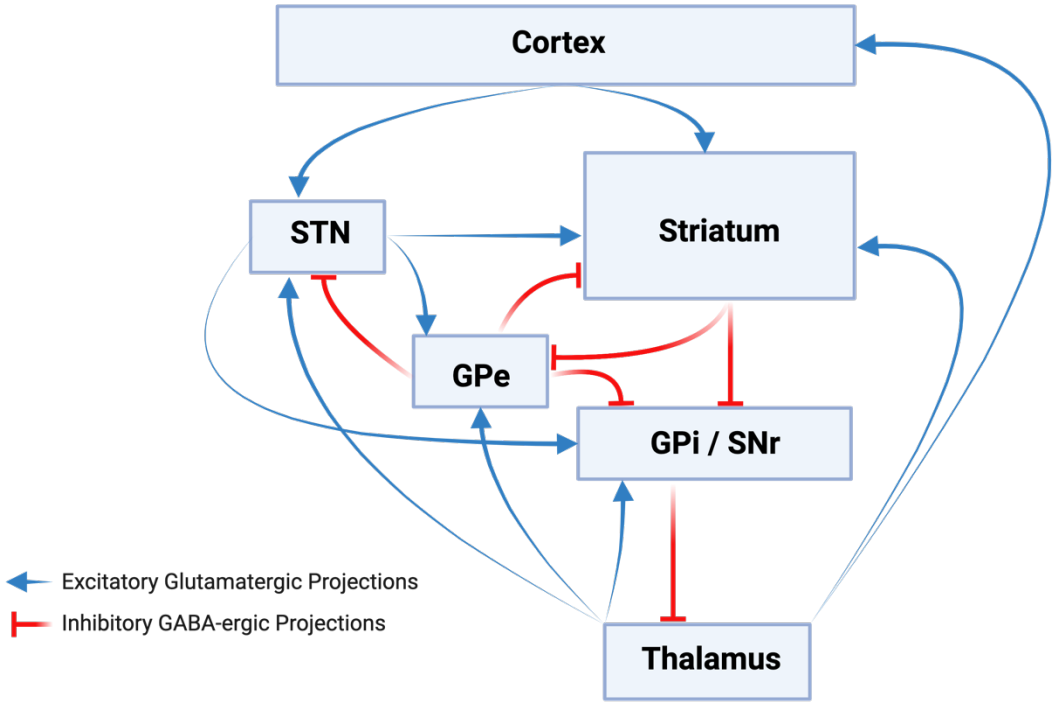

Figure 3: Cortico-Striatal-Thalamic Excitatory and Inhibitory Circuitry. The cortex, thalamus, and STN project glutamatergic afferents to multiple basal ganglia structures while the pallidum and striatum send GABAergic projections to several regions promoting an excitatory/inhibitory balance within the basal ganglia. Subthalamic Nucleus (**STN**), Globus Pallidus external (**GPe**), Globus Pallidus internal (**GPi**), Substantia Nigra Pars Reticulata (**SNr**). Created with BioRender.com.

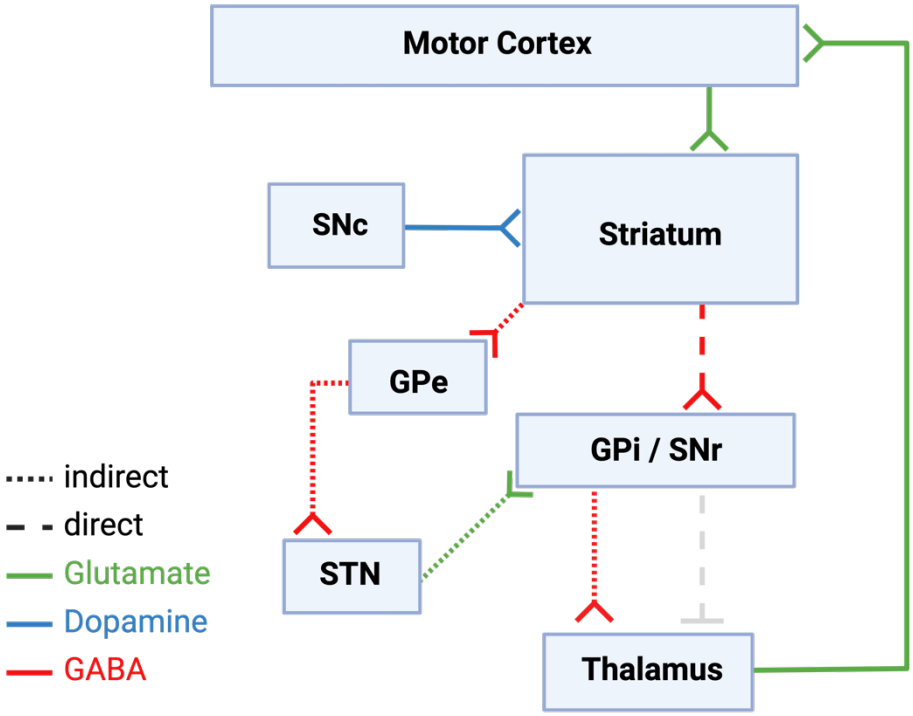

Figure 4: Cortico-Striatal-Thalamic Circuit Loop: Motor Modulation. dMSNs innervate the GPi/SNr directly disinhibiting thalamic activity (direct pathway) allowing an increase in glutamatergic drive in the cortex. iMSNs indirectly inhibit thalamic activity (indirect pathway) by innervating the GPe disinhibiting STN activity. STN activity increases GPi/SNr activity which inhibits thalamic activity, decreasing glutamatergic drive in the cortex. Green/Red/Blue (neurotransmitters), Subthalamic Nucleus (**STN**), Globus Pallidus external (**GPe**), Globus Pallidus internal (**GPi**), Substantia Nigra Pars Reticulata (**SNr**), Substantia Nigra Pars Compacta (**SNc**). Created with BioRender.com.

driving inhibition of activity and output. Inhibition of SNr/GPi drives disinhibition of the thalamus and increases thalamic activity (Lee et al., 2016), thereby increasing activity in cortical regions that mediate movement (Freeze et al., 2013).

In contrast to the actions of dMSNs, dopamine class-2 receptor (D2R)-containing MSNs (D2R-MSNs) indirectly modulate SNr/GPi activity via GPe innervation (**Figure 4**) (indirect pathway) (Gerfen & Bolam, 2010). Indirect pathway D2R-MSNs (iMSNs) innervate the GPe and inhibit the tonically active GABAergic neurons (**Figure 3, Figure 4**). Inhibition of GPe activity disinhibits the glutamatergic neurons within the STN. STN projections synapse onto the SNr/GPi, releasing the neurotransmitter glutamate (**Figure 3, Figure 4**), causing an increase in activity of the SNr/GPi. The SNr/GPi mainly projects to the thalamus (**Figure 3**), and the superior colliculus (Lee et al., 2011). Increased activation of the SNr/GPi increases inhibitory tone within the thalamus (Lee et al., 2016), decreasing thalamic glutamatergic drive to the cortex, thus resulting in decreased movement (Freeze et al., 2013).

One of the key components of voluntary movement regulation within the basal ganglia is the presence of the neuromodulatory neurotransmitter dopamine that arises from the SNc and innervates the striatum, synapsing on MSNs (Liang et al., 2008). The SNc consist of mainly a densely packed group of dopaminergic neurons that sends dopaminergic projections to the dorsal striatum (Sonne J, 2020). The SNc modulates striatal motor control and key components that influence movement such as procedural learning, shifting attention, and components of the temporal processing of learned interval-related tasks (Jahanshahi et al., 2006; Lanciego et al., 2012; Leisman et al., 2014).

Many classical diseases and disorders that exhibit motor dysfunction such as Parkinson's Disease (PD), Huntington's Disease, Autism Spectrum Disorder, Obsessive Compulsive Disorder (OCD), Substance Abuse, and Attention Deficit Hyperactivity Disorder (ADHD) have all been linked to perturbations in basal ganglia functional connectivity (Abdi et al., 2015; Daniela S. Andres & Olivier Darbin, 2018; Gremel & Lovinger, 2017; Subramanian et al., 2017; Welter et al., 2011). In the upcoming section we will discuss how improper basal ganglia function mediates, in part, motor dysfunction presented in a subset of the disorders listed above.

1.4 Basal Ganglia Mediated Motor Perturbations

Diseases/disorders listed in the previous section are commonly known to affect the basal ganglia, and these diseases are coupled with motor abnormalities. In addition, one of the classical characterizations of basal ganglia dysfunction is motor movement abnormalities (Ring & Serra-Mestres, 2002). Many basal ganglia-mediated movement perturbations manifest such that there is: an inability to facilitate appropriate/desired movements, for instance in PD, failure to prevent unwanted movements, like in OCD, or even something much more nuanced like psychostimulant substance abuse that displays a combination of both (Albin et al., 1989; DeLong, 1990; Gremel & Lovinger, 2017; Mink, 1996; Natarajan & Yamamoto, 2011). A prevailing thought within the basal ganglia field is that investigating the manifestation of motor perturbations associated with certain disease states can undoubtedly give rise to novel mechanistic insights that will aid in disease modifying therapeutics. We will briefly discuss our current understanding of how

the basal ganglia may mediate a subset of diseases/disorders with distinct motor abnormalities.

1.4.1 Parkinson's Disease

Parkinson's Disease (PD) is characterized by the loss of dopaminergic neurons innervating the basal ganglia (Neumann et al., 2018; Redgrave et al., 2010). A pathological hallmark is the presence of α-synuclein-containing Lewy bodies which accumulate in midbrain structures such as the SNc (**Figure 2**). A classical movement-related PD clinical feature is bradykinesia, which manifests as a reduction in movement amplitude and speed along with difficulty in initiating a motor sequence (McGregor & Nelson, 2019; Redgrave et al., 2010). The loss of dopaminergic neurons from the SNc drives aberrant dopaminergic signaling, which perturbs striatal signaling to other basal ganglia structures and is thought to mediate the primary motor symptoms presented in PD (McGregor & Nelson, 2019). Abnormal cellular and circuit function within the basal ganglia have been reported in several models of PD. Neuronal firing rates in the STN, striatum, and globus pallidum have all been reported to change in either frequency and/or pattern in parkinsonian models.

In parkinsonian models, there are increased neuronal firing rates within the STN (Benazzouz et al., 2002). In the globus pallidum, there are changes in both the firing pattern and frequency. Neurons within the GPe demonstrate a decrease in firing rate and asynchronous patterns, whereas neurons within the GPi exhibit aberrant bursting activity in human PD patients and/or in animal models of PD (Mallet et al., 2012; Mallet et al., 2008; Muralidharan et al., 2016; Soares et al., 2004; Starr et al., 2005). In the striatum,

both iMSNs and dMSNs have bidirectional changes in firing rates, such that iMSNs increase in Parkinsonian models when compared to healthy controls, while dMSNs decrease in firing rates when compared to healthy controls (Kita & Kita, 2011; Ryan et al., 2018; Sagot et al., 2018). The blunting and/or loss of dopaminergic signaling within the striatum drives perturbations in other basal ganglia structures, as the striatum sends projections to the globus pallidum (**Figure 4**). In addition, the interconnectedness in the circuity of the striatum, STN, and globus pallidum (**Figure 3**) demonstrate just how perturbations in the striatum could affect the activity other regions. In the upcoming sections, we will discuss how aberrant dopaminergic signaling can alter neuronal transmission within the striatum.

1.4.2 Obsessive Compulsive Disorder

Obsessive Compulsive Disorder (OCD) has been defined as a neuropsychiatric disorder that manifests as repetitive ruminations and/or stereotyped physical routines coupled with extreme anxiety (Ahmari et al., 2013; Burguière et al., 2015; Graybiel, 2008; Graybiel & Grafton, 2015; Schilman et al., 2010). Conventionally, there is evidence to suggest that in OCD pathology, the CST circuitry loop is dysregulated (Ahmari et al., 2013; Saxena et al., 2001). Using the CST model (**Figure 4**) we see the net effect of competing motor programs (direct vs indirect), in that the direct pathway drives hyperkinetic motor behavior and the indirect pathway drives hypokinetic motor behavior (Mink, 1996); during presentations of repetitive/stereotyped routines, the direct pathway is plausibly overactive. Interestingly, humans with Huntington's Disease (HD) have a higher prevalence of also presenting with OCD like symptoms in the early stages

of HD (Beglinger et al., 2007). In fact, there have been studies indicating that some individuals with OCD were first diagnosed with Huntington's disease (Scicutella, 2000). Both the chorea that we observe in Huntington's and the repetitive nature of inappropriate movements, such as tics, present in OCD phenotypes involve a failure to prevent unintended motor behaviors. However, it is very difficult to fully understand the pathophysiology of OCD in humans, as imaging studies can provide some information but lack mechanistic certainty and cell type specificity.

Animal grooming models are therefore exemplary translational models for repetitive behavior dysfunction. Rodent self-grooming involves repetitive stereotyped motor behaviors (Kalueff et al., 2016). In a subset of OCD populations, pathological self-grooming is a key clinical presentation (Ahmari et al., 2013; Kalueff et al., 2007; Kalueff et al., 2016). In rodent models, the striatum is a focal point for OCD-like phenotypes as the striatum mediates the execution of complete motor sequences, and mechanistic insight into how the striatum regulates such behavior could be promising for translational interventions into repetitive motor presentations associated with OCD-like phenotypes (Ahmari et al., 2013; Kalueff et al., 2007; Kalueff et al., 2016; Shmelkov et al., 2010). Interestingly, self-grooming can be modulated pharmacologically using dopamine D1 receptor agonists or dopamine D2 receptor antagonists (Berridge & Aldridge, 2000; Kalueff et al., 2016; Taylor et al., 2010). Moreover, D1 receptor-lacking mice have disruptions in self-grooming bouts and micro-grooming sequences (Cromwell et al., 1998). Another line of evidence that implicates dopaminergic signaling being an obligatory player in mediating OCD-like phenotypes is dopamine transporter (DAT) genetic knockdown mice (DAT-KD). DAT-KD causes hyper-dopaminergic tone within

myriad brain regions and behaviorally we see an increase in what researchers have deemed a super-stereotypy (Berridge & Aldridge, 2000; Berridge et al., 2005) Furthermore, striosomes (chemically compartmentalized areas/ 'striatal bodies') within the striatum are highly activated after states of hyper-dopaminergic tone, the increased activation of striosomes has been linked to increases in repetitive motor behavior (Canales & Graybiel, 2000; Crittenden & Graybiel, 2011; Kalueff et al., 2016). In addition, striatal dMSNs and iMSNs contain D1- or D2-dopamine receptors respectively. Taken together, aberrant dopamine signaling may disrupt the activity balance in dMSN and/or iMSN function which may mediate, in part, OCD-like phenotypes.

1.4.3 Psychostimulant Addiction

Psychostimulant drugs such as amphetamine-type stimulants (amphetamine and methamphetamine) and cocaine were ranked second as the most used illicit drugs globally according to a 2011 report released by United Nations Office on Drugs and Crime (UNDOC). Furthermore, in the United States, the Center for Disease Control and Prevention reported a 37% increase in psychostimulant overdoses from 2016 to 2017. Although psychostimulant use is widespread and overdoses are increasing, unlike opioid addiction there are no pharmacological interventions, such as methadone maintenance programs, meeting the conditions for regulatory approval that may improve outcomes in individuals with psychostimulant substance use disorders (Phillips et al., 2014).

Currently, the primary intervention for psychostimulant addiction is cognitive behavioral therapy approaches. Pharmacological interventions have been challenging and have remained stagnant due, in part, to the inadequate understanding of the

pathophysiology and lack of knowledge of specific neuronal substrates that mediate the disease/disorder (Phillips et al., 2014). Myriad evidence suggests that psychostimulants' central action is aberrant modifications to dopaminergic neurotransmission and perturbations in glutamatergic neuroplasticity (Dela Peña et al., 2015; Kalivas, 2007; Rebec, 2006). One of the prominent effects of psychostimulants is the production of increased motor behaviors (Asser & Taba, 2015). In addition, the increase in abnormal transient motor behaviors has been directly linked to increases in dopaminergic tone within the striatum (Asser & Taba, 2015). The dopaminergic neuron terminals that synapse on GABAergic MSNs within the striatum are postulated to mediate many of the reinforcing and behavioral effects of psychostimulants through dysregulated release of the dopamine neurotransmitter (Lobo & Nestler, 2011; Rebec, 2006; Salery et al., 2020; Yager et al., 2015). Much of the research conducted using psychostimulants and observing the striatum is done within the ventral striatum; however, there is also evidence that psychostimulants alter dendritic spine dynamics such as shape and density within the dorsal striatum (Jedynak et al., 2007). In addition, chronic psychostimulant exposure gives rise to enhanced dorsal striatum activity (Schneck & Vezina, 2012). Taken together, the dorsal striatum is a key brain region in which psychostimulants exert their effects. A better understanding of how psychostimulants alter dorsal striatal MSN function may uncover novel, disease-modifying therapeutics that will allay the deleterious social and personal effects of psychostimulant abuse/addiction. In the upcoming section we will further examine striatal anatomy and biochemistry as detailing the organization of the striatum will provide mechanistic insight into how the striatum

mediates movement. This understanding can act as a bridge to determining how diseases such as psychostimulant misuse/addiction manifest.

1.5 Striatal Anatomy and Movement

One chief commonality amongst the different disease states discussed above is that the disease-induced motor perturbations are in part due to striatal dysfunction. Understanding striatal anatomy and function is essential in understanding the pathophysiology of the disease states discussed earlier. Here we discuss key striatal anatomy features and function that contribute to movement. The functional anatomy of the striatum can be subdivided into the ventral and dorsal striatum. The ventral striatum can be further divided into the nucleus accumbens (NAc) with sub-divisions (core and shell), and the olfactory tubercle (OT). The dorsal striatum is subdivided into the dorsal medial striatum (DMS) and the dorsal lateral (DLS) striatum. The subdivisions within the dorsal striatum are postulated to control various functions; the DMS mainly mediates associative tasks thereby modulating goal-directed behavior, whereas the DLS controls sensorimotor function mediating habitual motor actions (Balleine & O'Doherty, 2010; Burton et al., 2015; Stalnaker et al., 2012). Together, the dorsal striatum is critical for decision making, and is paramount in delineating how the striatum mediates/influences movement. Furthermore, neurodegenerative diseases with major movement perturbations, PD and HD, the dorsal striatum is a critical brain region. Specifically, loss of dopaminergic innervation in PD begins in loss of neurons from the SN that innervate the dorsal striatum. Moreover, neurodegeneration present in HD happens mainly in the dorsal striatum (Burrus et al., 2020; Chen et al., 2020; Fernández-García et al., 2020; Hanganu

et al., 2015; Kish et al., 1988). We will now examine the sub-populations of MSNs (dMSNs and iMSNs) and how modulation of their function can impact striatal mediated motor behaviors.

1.6 Medium Spiny Neurons

MSNs are the principal neurons in the striatum. As their name implies, MSNs contain an abundance of dendritic spines, which contain organized synaptic signaling components to respond to presynaptic glutamate release. Intrinsic signaling at MSN dendritic spine synapses drive striatal mediated behaviors (Kreitzer & Malenka, 2008). Glutamatergic inputs arising from the cortex and dopaminergic inputs arising from the SN synapse on striatal MSN dendritic spines and are major contributors to striatal MSN signaling. MSNs are subdivided into two classes: D1R-containing dMSNs and D2R-containing iMSNs and are associated with increased motor activity when stimulated (Korchounov, 2008; Waszczak et al., 2002). Molecular characterization of striatal MSNs is one of the most efficient ways to distinguish the two MSN populations. Molecular markers such as D1R (*Drd1*), dynorphin (*Pdyn*), and substance P (*Tac1*) are enriched in and used as markers of dMSNs; while the D2R (*Drd2*), enkephalin (*Penk*), and adenosine 2a receptors (*Adora2a*) are enriched in and used as markers of iMSNs (Gerfen, 1992; Gerfen et al., 2013; Heiman et al., 2008). Both dMSNs and iMSNs are in all anatomical domains of the striatum and are amalgamated in such a way that there are no true physical demarcations that would differentiate between the two neuronal populations. However, recently there is growing evidence that may suggest there are some distinct morphological combined with electrophysical features that may parse out some

differences, such as dendritic length and organization, with iMSNs having smaller dendritic trees (Gagnon et al., 2017; Gertler et al., 2008). Furthermore, it is postulated that the morphological differences coupled with differential molecular signaling parameters may explain the electrophysiological differences found between dMSNs and iMSNs mediating differential plastic responses from each cell-type. For example, iMSNs are basally more excitable (Ding et al., 2008; Gertler et al., 2008; Kravitz et al., 2010). Molecular contributions to the electrophysiological differences between dMSNs and iMSNs were thought to be due to differential modulation of signaling downstream of muscarinic-1 receptor (M_1R) activation (Shen et al., 2007). The activation of cholinergic interneurons stimulates M_1Rs in both MSN populations; however, downstream signaling of M_1Rs downregulates inward rectifier potassium family, subtype 2 (Kir2) channels within iMSNs, but no change in dMSNs (Shen et al., 2007). However, research now suggests that changes in M_1R activation alone do not completely explain the disparities between dMSN and iMSN excitability (Gertler et al., 2008). The evidence suggesting dichotomous function between dMSNs and iMSNs beyond differential connectivity and discrepancies in molecular changes such as the regulation of Kir2 channels is still unclear. However, appropriate striatal function requires competent molecular signaling from both iMSNs and dMSNs. To ensure competent signaling, MSN post-synaptic signaling molecules must be able to respond appropriately to environmental change such as neurotransmitter release. Specifically, signaling molecules must be temporally and spatially organized to appropriately propagate downstream signaling that drives or inhibits MSN activity. In the upcoming section we will discuss post-synaptic signaling molecules within striatal MSNs that contribute to MSN activity.

1.6.1 Striatal MSN Post-synaptic Signaling Molecules

Proper post-synaptic neuronal signaling is maintained by the targeting of specific kinases and phosphatases within striatal MSNs regardless of sub-type. Mammalian kinases add a phosphate group (PO_4) on the polar side chain of serines, threonines, and tyrosines at specific consensus sites whereas phosphatases remove the PO_4. Subsequently, adding the PO_4 switches the hydro-polarity of said protein to a more hydrophilic polarity, in turn driving conformational changes which allow the phosphorylated protein to interact with additional molecules (Ardito et al., 2017). Myriad protein substrates have kinase- and phosphatase-specific sites that can bidirectionally modulate said protein function, giving rise to molecular plasticity via a process known as reversible protein phosphorylation. Within the post-synapse these changes mediate synaptic plasticity(Woolfrey & Dell'Acqua, 2015). Here we will examine how coordination of phosphorylation states can influence signaling dynamics of myriad post-synaptic proteins within striatal MSNs. Protein kinases and phosphatases work synergistically to mediate multifaceted molecular signaling responses at targeted substrates. Specifically, reversible protein phosphorylation can impact the magnitude, timing, or localization of a specific molecular response. The balance of kinase and phosphatase activity gives rise to appropriate plastic cellular responses. Moreover, while not always the case, many post-synaptic proteins are maintained in a dephosphorylated state due to higher levels of basal phosphatase activity, whereas glutamatergic and dopaminergic signaling can shift the balance towards kinases which promote phosphorylation and activity of specific substrates. In striatal MSNs, both glutamatergic and dopaminergic signaling contribute greatly to striatal synaptic plasticity.

1.6.2 Glutamatergic Signaling within Striatal MSNs

Glutamatergic afferents from both the thalamus and the cortex (**Figure 3**) synapse on the dendritic spines of striatal MSNs. Glutamatergic synaptic transmission is facilitated by ionotropic glutamate receptors N-methyl-D-aspartate (NMDA)- and AMPA receptors (NMDARs and AMPARs, respectively) localized to the post-synaptic density (PSD) of the synapse. Functional NMDARs are heterotetramers that consist of at least two obligatory GluN1subunits and two GluN2 subtype (GluN2A-D) or two GluN3 subtype (GluN3A/B) subunits (Karakas & Furukawa, 2014; Paoletti et al., 2013). AMPARs become functional with the assembly of either hetero- or homotetramers composed of four subunits (GluA1-4). NMDARs and AMPARs play critical roles in modulating MSN dendritic spine synaptic strength. Precisely, excitatory synapses can undergo long-lasting alterations via molecular neuroadaptations in response to either sustained or diminished glutamatergic synaptic transmission, forms of synaptic plasticity defined as either long-term potentiation (LTP) or long-term depression (LTD) respectively (Bear & Malenka, 1994; Malenka & Bear, 2004; Woolfrey & Dell'Acqua, 2015). Furthermore, in the striatum, LTD and LTP are strongly correlated with motor learning and performance in the dorsal striatum (Giordano et al., 2018; Hawes et al., 2015; Perrin & Venance, 2019; Rothwell et al., 2015; Xiong et al., 2015; Yin et al., 2009). Specifically, in the DMS, in which researchers trained mice on a T-maze, LTP but not LTD was present in the early phase of skill acquisition, while LTD was present during the late phase (learned skill switches to optimization of performance) in the DLS (Hawes et al., 2015). In addition, increased and decreased AMPA/NMDA ratios are a hallmark correlate of synaptic plasticity as this phenomena is characterized by changes in

the surface expression and modulation of synaptic transmission by glutamatergic receptors (Kerchner & Nicoll, 2008). Indeed, synaptic plasticity within the striatum appears to be task and region specific (Rothwell et al., 2015; Shan et al., 2014). In addition, within the DMS the acquisition of goal directed tasks was shown to increase AMPA/NMDA ratios in dMSNs, while decreasing AMPA/NMDA ratios in iMSNs (Shan et al., 2014). In addition, in a task in which subjects had to learn serial tasks, the ability to learn the sequencing order correlated with the increase of AMPA/NMDA ratios in the DLS (Rothwell et al., 2015). Together striatal mediated behaviors depend on appropriate glutamatergic signaling. Many of the changes in glutamatergic transmission are meditated by downstream signaling of kinases and phosphatases, whose activation was initiated by glutamatergic signaling. For example, when NMDARs are stimulated by glutamate and its co-agonist, glycine or d-serine, calcium (Ca^{2+}) (a critical second messenger) enters the MSN and can activate kinases such as Ca^{2+}/calmodulin (CaM)-dependent protein kinases (CaMKs) and protein kinase C (PKC), along with phosphatases such as calcineurin (PP2B) (**Figure 5**) (Rajadhyaksha et al., 1998). These signaling molecules go on to phosphorylate or dephosphorylate additional proteins within the MSN (**Figure 5**), including AMPARs which can further enhance glutamatergic synaptic transmission or decrease transmission (respectively). The phosphorylation of AMPARs within the striatum has been well characterized. Specifically, phosphorylation at serine 845 (S845), a protein kinase A (PKA) site, and serine 831 (S831), a CaMKII and PKC site, on the GluA1 subunit modulate AMPAR function (Jenkins & Traynelis, 2012; Xue et al., 2017). Specifically, phosphorylation of S845 and S831 has been shown to promote glutamatergic

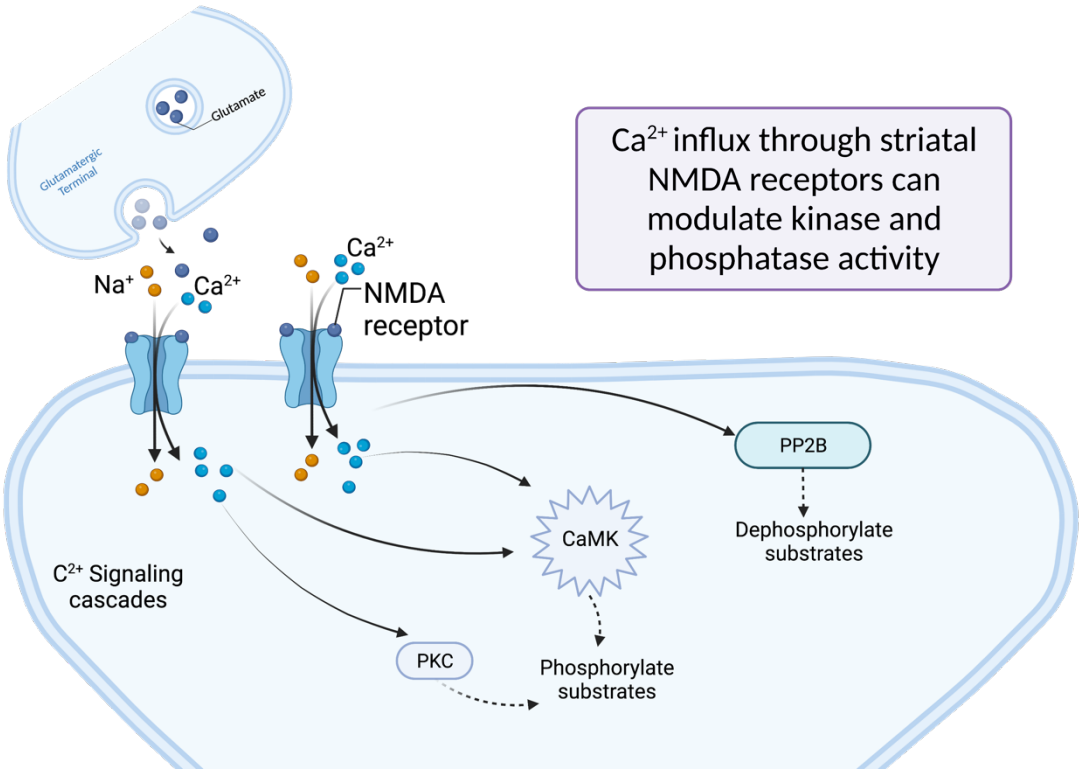

Figure 5: NMDA Receptors Modulate Kinase and Phosphatase Activity Through Ca^{2+} Influx. Simple schema demonstrating how prominent kinases such as PKC and CaMK and phosphatase, PP2B are regulated via Ca^{2+} influx through NMDA receptors. Created with BioRender.com.

transmission through increased surface expression via trafficking and enhanced conductance (Carvalho et al., 2000; Jenkins & Traynelis, 2012; Wang et al., 2005). Moreover, these sites are extremely important for striatal function as GluA1 subunits are found on all MSNs within the striatum (Bernard et al., 1997; Xue et al., 2017). These data taken together explicitly demonstrate that glutamatergic transmission is a critical regulator of striatal function. In addition, dysregulated phosphorylation states of NMDA and AMPA receptors have been implicated in several disease states associated with striatal dysfunction (Kalivas et al., 2009; Ohtsuka et al., 2008; Paoletti et al., 2013; Ravenscroft & Brotchie, 2000).

1.6.3 Dopaminergic Signaling within Striatal MSN

D1Rs and D2Rs are highly abundant dopamine subtypes within the striatum. MSNs expressing D1Rs (dMSNs) or expressing D2Rs (iMSNs) are correlated with striatonigral (direct pathway) or striatopallidal (indirect pathway), respectively, output neurons (Xue et al., 2017). The dopamine receptors are G protein-coupled receptors, in which D1Rs are coupled to ($G_{\alpha s}$) and D2Rs are coupled to ($G_{\alpha i/o}$) which modulates protein kinase A (PKA) activity through their production or inhibition of cAMP, respectively. Specifically, D1Rs promote cAMP production whereas D2Rs limit cAMP production, via increasing or decreasing adenylyl cyclase activity, respectively (Creese et al., 1983; Surmeier et al., 2010; Xue et al., 2017). It is thought that in many striatal-mediated disease states (e.g. PD, HD, Psychostimulant Abuse/Addiction), aberrant neuroadaptations within the striatum caused by alterations in dopaminergic and glutamatergic signaling are the prominent causes of classical motor abnormalities

observed. Dopaminergic and glutamatergic signaling dysfunction physically manifest as motor perturbations, and the motor deficits gradually exacerbate in correlation to dopaminergic tone within the striatum as either diminished (e.g. PD) or excessive (e.g. psychostimulant-induced hyperlocomotion) motor outputs (Bernheimer et al., 1973; Rajadhyaksha et al., 1998). Interestingly, dopaminergic signaling within the striatum can also directly or indirectly reinforce synaptic changes via modification of glutamatergic transmission though downstream signaling cascades (**Figure 6**). Activation of D2Rs is essential for high frequency stimulation-induced (HFS)-LTD by enhancing the retrograde endocannabinoid signaling pathway which limits presynaptic glutamate release, indirectly decreasing post-synaptic glutamatergic transmission (Calabresi et al., 1997; Kreitzer & Malenka, 2005; Tang et al., 2001; Yin & Lovinger, 2006). In addition, D2 stimulation can drive decreases in AMPA receptor currents by attenuating NMDAR signaling (Cepeda et al., 1993; Hernández-Echeagaray et al., 2004; Nieto Mendoza & Hernández Echeagaray, 2015). Furthermore, activation of D1Rs increases PKA signaling which targets phosphorylation of AMPAR GluA1 subunit at S845 (Allen et al., 2006; Price et al., 1999; Snyder et al., 2000; Xue et al., 2017). In addition, blocking D1Rs in the dorsal striatum permits endocannabinoid mediated LTD which attenuates glutamatergic transmission through blocking presynaptic glutamate release (Shen et al., 2008). In addition, dopaminergic and glutamatergic transmission can converge on single molecules driving reversible phosphorylation. For instance, within the striatum, the phosphorylation or dephosphorylation of dopamine and cyclic AMP-regulated phosphoprotein of 32 kDa (DARPP-32) at threonine 34 (Thr34) is regulated by D1R signaling and NMDAR signaling. Specifically, increases in PKA signaling via D1R activation can promote

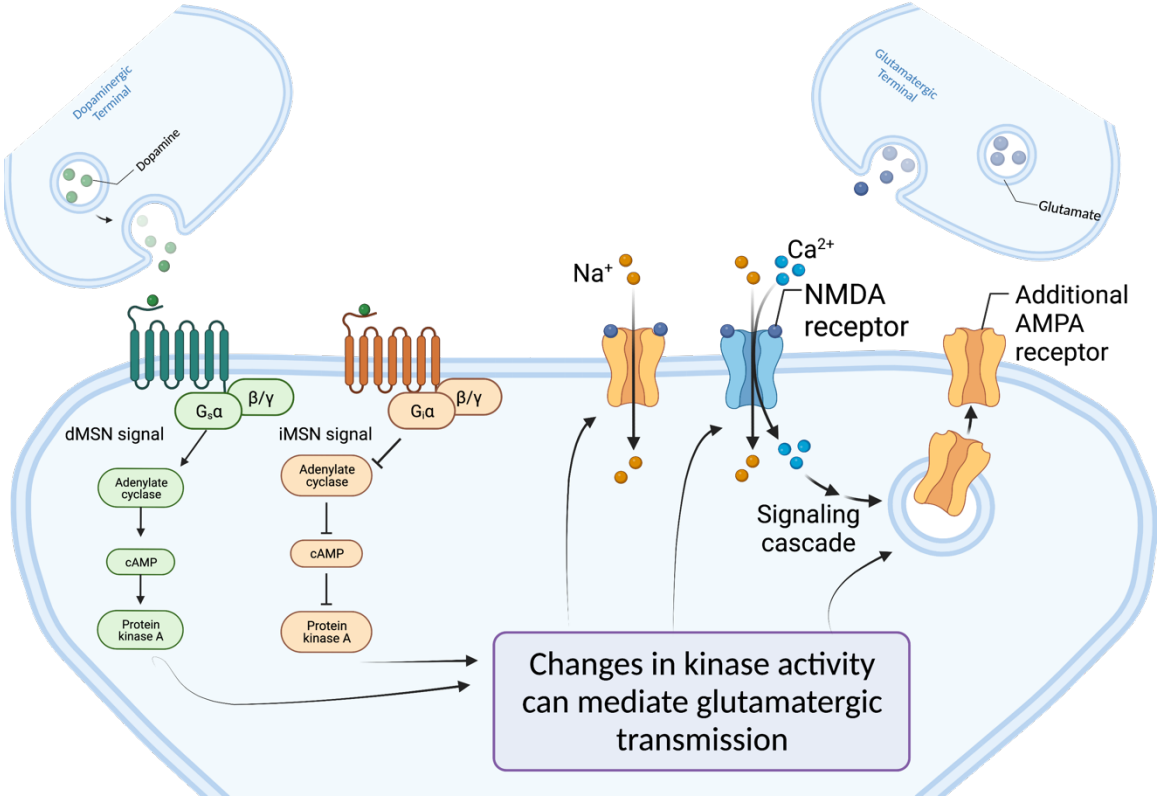

Figure 6: Dopaminergic Signaling Mediates Glutamatergic Transmission via Kinase Activity. Here we show that dopaminergic signaling can modulate glutamatergic transmission via changes in kinase activity. Phosphorylation changes in glutamatergic receptors can drive receptor transmission dynamics via trafficking and conductance. Created in BioRender.com.

phosphorylation of DARPP-32 at Thr34, creating a potent PP1 inhibitor and furthering PKA signaling actions, while activation of NMDARs can drive dephosphorylation by increased calcineurin activity at the same Thr34 site on DARPP-32 (**Figure 7**) (Greengard et al., 1999; Lindskog et al., 2006; Nishi et al., 2017; Yger & Girault, 2011). The Thr34 site on DARPP-32 is incredibly important in striatal function as it is a potent inhibitor of protein phosphatase 1 (PP1) which is a critical regulator of MSN synaptic signaling (Greengard et al., 1999). PP1 is a major Ser/Thr phosphatase within the dendritic spines of MSNs and dephosphorylates myriad proteins that mediate glutamatergic and dopaminergic transmission, and dendritic spine formation and shape (Allen et al., 1997; Colbran et al., 1997; Foley et al., 2021; Ouimet et al., 1995; Strack et al., 1999).

Interestingly, Ser/Thr phosphatases such as PP1 are promiscuous and associate with targeting proteins to attain substrate specificity which prevents indiscriminate dephosphorylation of interacting proteins (Heroes et al., 2013). Specifically, PP1 has ~200 interacting proteins, making the regulation of PP1activity critical for proper function (Bollen et al., 2010; Heroes et al., 2013). The major targeting protein for PP1 in MSN dendritic spines is spinophilin, a neuronal scaffolding protein (Allen et al., 1997; Colbran et al., 1997). Moreover, spinophilin is required for proper glutamatergic synaptic transmission and dendritic spine morphology due to its interaction with actin and PP1 (Feng et al., 2000). In the next section we will examine spinophilin's function within the striatum.

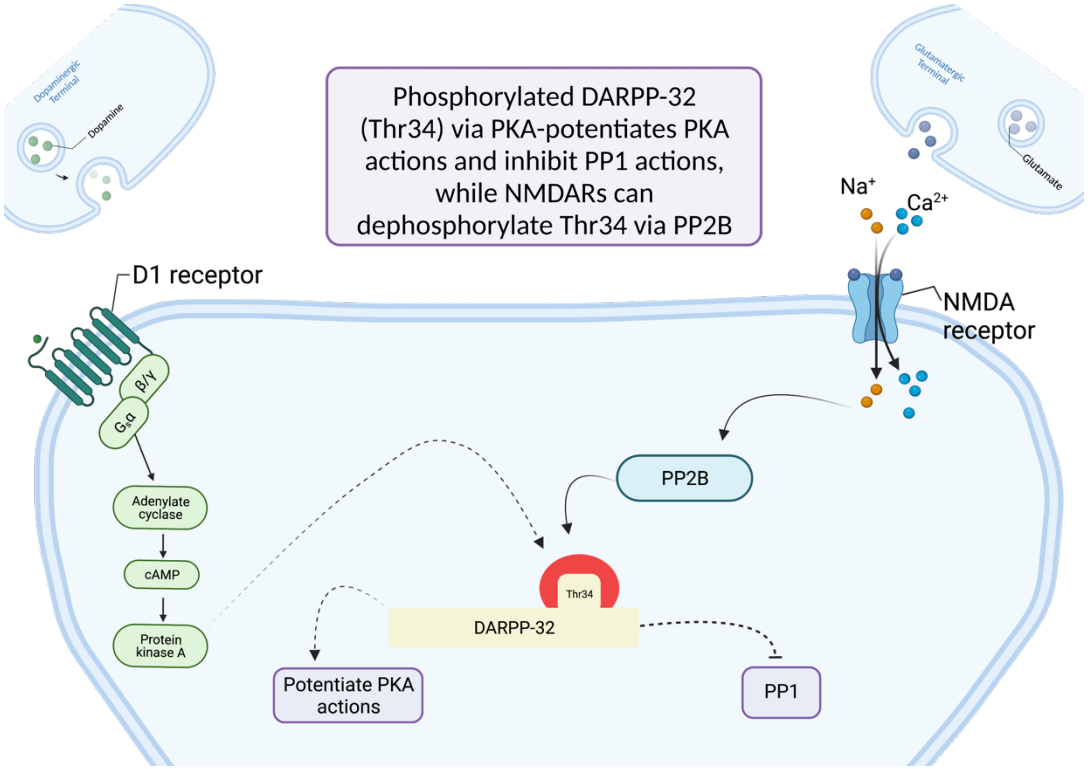

Figure 7: Reversible Phosphorylation Through Dopaminergic and Glutamatergic Signaling Convergence on DARPP-32. Stimulation of dMSNs increase PKA signaling which can phosphorylate DARPP-32 at Thr34, inhibiting PP1 and further potentiating PKA signaling. Ca^{2+} influx through NMDARs increase calcineurin activity which dephosphorylates DARPP-32 at Thr34. Created in BioRender.com

1.7 Striatal Spinophilin Molecular Function

The *PPP1R9B* (spinophilin) gene consists of 10 exons and is positioned on chromosome 17 at 17q21.33, a region enriched with tumor suppressor genes (Porter et al., 1994; Sarrouilhe et al., 2006; Verdugo-Sivianes & Carnero, 2022). Indeed, spinophilin was found to function as a tumor suppressor gene (Verdugo-Sivianes & Carnero, 2022). We will not focus spinophilin's tumor suppressing function, but instead on how spinophilin functions within the CNS.

Neurabin-2, better known as spinophilin was first described by multiple research groups in the late 1990s. One group identified a novel PP1 targeting protein that was enriched in the PSD of dendritic spines, thus the moniker 'SPINO-philin' (Allen et al., 1997). Other groups independently recognized an actin-binding protein that was similar to another protein, neurabin-1, that was enriched in the PSD of rat brain and at adherens junctions within the rat liver (MacMillan et al., 1999; Satoh et al., 1998). Briefly, neurabin-1 is considered a homolog of spinophilin, it also acts as a PP1 targeting protein and is highly expressed in dendritic spines (Kelker et al., 2007; MacMillan et al., 1999; E. Chris Muly et al., 2004). Spinophilin was found to be expressed in myriad brain regions such as the olfactory blub, hippocampus, cerebellum, striatum, olfactory tubercle, thalamus, hypothalamus, and cortex, with a particularly high concentration in dendritic spines that had excitatory synapses, like that of the dendritic spines of striatal MSNs (Allen et al., 1997; Ouimet et al., 1995; Sarrouilhe et al., 2006; Satoh et al., 1998). Spinophilin was also found in prefrontal cortex of primates, specifically macaques, demonstrating again that spinophilin is highly concentrated in the PSD of dendritic spines at excitatory synapses (E. C. Muly et al., 2004). Spinophilin was also found to be

expressed in other organs such as the lungs and pancreas (Allen et al., 1997; Ruiz de Azua et al., 2012).

1.7.1 Actin Binding Domains

Spinophilin is a highly conserved, multi-modular domain, neuro-scaffolding protein that consist of 817 amino acids (aa) (**Figure 8**) (Sarrouilhe et al., 2006; Verdugo-Sivianes & Carnero, 2022). At the N-terminal region, spinophilin has two F-actin binding domains from 1-154aa (actin-binding D1) and 164-282aa (actin-binding D2) (**Figure 8**) in which there are multiple kinase consensus sequences that enable spinophilin phosphorylation, modulating spinophilin-actin interactions. Phosphorylation at Ser100, and Ser116 by CaMKII (**Figure 8**) reduces spinophilin's ability to bind F-actin (Grossman et al., 2004). When spinophilin is phosphorylated at Ser97 by PKA (**Figure 8**), the phosphorylated spinophilin was found to be in greater abundance within the cytosol and less likely to be in the PSD using subcellular fraction (Hsieh-Wilson et al., 2003). Moreover, a study found the stoichiometry between spinophilin-actin interaction to be greatly reduced when Ser97 was phosphorylated by PKA suggesting a disruption spinophilin-actin interaction (Hsieh-Wilson et al., 2003). It has also been demonstrated that spinophilin within the actin-binding domain can be phosphorylated by ERK2 at Ser15 and S205 and by cyclin-dependent protein kinase-5 (CDK5) at Ser17 (**Figure 8**) (Futter et al., 2005). The modulation of spinophilin-actin interactions can determine where the spinophilin-PP1 complex is anchored within the spine, having implications in synaptic signaling, and dendritic spine morphology (Futter et al., 2005; Grossman et al., 2004; Hsieh-Wilson et al., 2003).

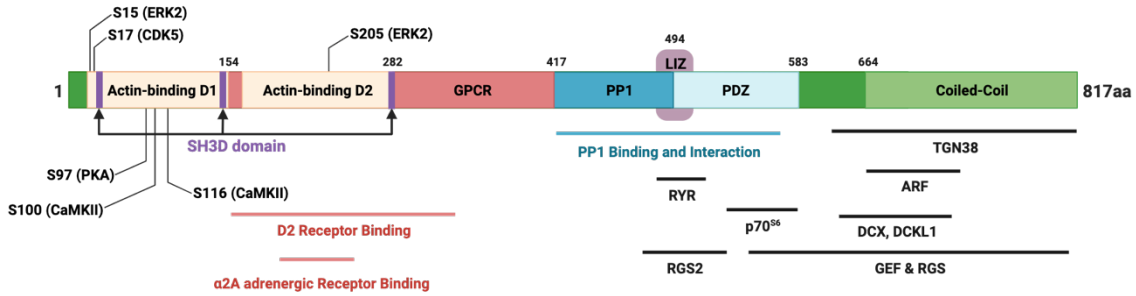

Figure 8: Spinophilin Modular Domains and Interacting Proteins Schema. Simple schema of spinophilin's modular domains, motifs, and protein interactions. Illustration adapted from (Carnero, 2012; Sarrouilhe et al., 2006; Verdugo-Sivianes & Carnero, 2022). Created in BioRender.com.

1.7.2 SH3 Binding Domain

Spinophilin also contains three proline rich regions that create a Src homology 3 (SH3) binding domain spanning 8-14aa, 137-143aa and 281-287aa (**Figure 8**) (Allen et al., 1997; Mayer & Eck, 1995; Sarrouilhe et al., 2006). Interestingly, very little research has been done on the functional role having a SH3 binding domain plays on spinophilin. However, a recent study did identify spinophilin as an interacting protein with the Nervous wreck (Nwk) protein in *Drosophila* using mass spectrometry (Ukken et al., 2016). Moreover, Nwk is a F-BAR protein that binds to SH3 domains and mediates synaptic growth and physiology (Ukken et al., 2016).

1.7.3 G-protein Coupled Receptor Binding Domain

Spinophilin has a G-protein coupled receptor (GPCR) binding domain positioned at 151-444aa (**Figure 8**) that interacts with the third intracellular (3i) loop of D2 receptors and alpha2A (α2A) adrenergic receptors (Richman et al., 2001; Smith et al., 1999). Spinophilin also co-immunoprecipitates μ opioid receptors (MORs) (Charlton et al., 2008), β-cell M(3) muscarinic acetylcholine receptors (M3Rs) (Ruiz de Azua et al., 2012), and metabotropic glutamate receptor subtype 5 (mGLUR5) receptors (Di Sebastiano et al., 2016; Morris et al., 2018) We will explore spinophilin's function in D2 containing MSNs in the upcoming chapters.

1.7.4 PDZ Binding Domain

Spinophilin also contains a PSD-95/Discs Large/ZO-1 (PDZ) binding domain that stretches from 492-583aa (**Figure 8**) (Sarrouilhe et al., 2006; Verdugo-Sivianes &

Carnero, 2022). PDZ domains are one of the most common interaction domains for transmembrane receptors like AMPARs and NMDARs and synaptic proteins within the PSD ((Erlendsson et al., 2019; Ye & Zhang, 2013; Zhu et al., 2016). Spinophilin was shown to interact with both NMDA and AMPA receptors via the PDZ domain (Kelker et al., 2007).

1.7.5 Leucine/isoleucine Zipper and Coiled-coil Domain

Spinophilin has a leucine/isoleucine zipper (LIZ) that forms coiled coils stretching from 485-510aa (**Figure 8**) (Sarrouilhe et al., 2006; Verdugo-Sivianes & Carnero, 2022). The LIZ region is an alpha helical motif that allows for the targeting of phosphatases such as PP1 to other proteins such as ion channels (Kass et al., 2003; Sarrouilhe et al., 2006). In addition, spinophilin also has a coiled-coil domain at the carboxyl-terminus that is positioned from 664-814aa (**Figure 8**). The coiled-coil domain permits the dimerization of spinophilin with itself or its homolog neurabin-1 (Baucum et al., 2010; Sarrouilhe et al., 2006). In addition, the coiled-coil domain has been shown to interact with other post-synaptic proteins such as densin-180, CaMKII and doublecortin-like kinases (Baucum et al., 2012; Baucum et al., 2010; Shin et al., 2013; Verdugo-Sivianes & Carnero, 2022).

1.7.6 PP1 Binding Domain

Arguably one the most important binding regions on spinophilin is the PP1 binding domain that spans from 417-494aa (**Figure 8**). PP1 binding occurs through a RVxF motif and spinophilin has a pentapeptide motif sequence (R/K-R/K-V/I-X-F)

positioned between 447aa and 451aa that allows for PP1 binding (Peti et al., 2013; Sarrouilhe et al., 2006). However, there are other regions on spinophilin that bind to different regions of PP1, extending PP1 interactions from 417-583aa, increasing the type of protein interactions with PP1 (**Figure 8**) (Ragusa et al., 2011).

Functionally, PP1 mediates myriad cellular processes including transcription, mitosis, DNA repair, cytoskeleton organization, and synaptic transmission (Cannon, 2010; Foley et al., 2021; Oliver et al., 2002; Peti et al., 2013; Rebelo et al., 2015). PP1 has four different isoforms (PP1α, PP1β, PP1γ1 and PP1γ2) that are found in various tissues (Peti et al., 2013). Although all isoforms are found in the nucleus, the expression of PP1 isoforms can vary depending on the type of tissue or location within the cell. For example, PP1γ2 is exclusively found in the testes (Chun et al., 1994). In addition, PP1α, PP1β, and PP1γ1 are all found in neurons; however, PP1β has a greater abundance in the soma of a neuron whereas PP1α, and PP1γ1 have a higher abundance in the dendritic spine (MacMillan et al., 1999; Terry-Lorenzo et al., 2002).

1.8 Striatal Spinophilin Function and MSN Synaptic Plasticity

Changes in post-synaptic protein expression, protein-protein interactions, and ionotropic/metabotropic receptor signaling within dendritic spines can modify MSN synaptic plasticity both structurally and molecularly. Indeed, spinophilin can regulate dendritic spine morphology through its interactions with actin, which is modulated by several phosphorylation sites within the actin-binding domain of spinophilin (**Figure 8**) (Feng et al., 2000; Verdugo-Sivianes & Carnero, 2022). Moreover, loss of spinophilin promotes increased glutamatergic synaptic transmission presumably due to blunted PP1

targeting of specific AMPA and NMDA subunits (Feng et al., 2000; Westphal et al., 1999; Yan et al., 1999). In addition, loss of spinophilin drives deficits in cortico-striatal HFS-LTD and is rescued by the D2R agonist, quinpirole (Allen et al., 2006). Interestingly, spinophilin has been shown to directly interact with D2Rs on the 3i loop (Smith et al., 1999). These data taken together position spinophilin as a key mediator in dopaminergic and glutamatergic signaling, and the regulation of other synaptic protein interaction events that occur within the MSN mediating synaptic plasticity. Moreover, striatal mediated motor actions are facilitated, in part, by proper signaling within the striatal MSN (Kravitz & Kreitzer, 2012). Thus, understanding how spinophilin participates in striatal mediated motor actions is critical in understanding the motor perturbations exhibited in striatal mediated disease states.

1.9 Hypothesis

Given that 1) molecular signaling changes within dendritic spines, such as reversible phosphorylation, are paramount in maintaining and balancing striatal MSN function and synaptic plasticity, 2) phosphatases are gatekeepers for limiting protein phosphorylation, 3) spinophilin is the major PP1 targeting protein within the PSD of MSN dendritic spines, and 4) functional and structural plasticity within MSNs is mediated through spinophilin's interaction with PP1 and critical post-synaptic proteins, we hypothesize *that spinophilin is critical in facilitating striatal-mediated motor behaviors such as motor learning/performance and amphetamine-induced behavioral sensitization in a cell type-specific manner by promoting changes in protein substrate phosphorylation and protein expression in myriad post-synaptic striatal molecules.*

Chapter II: Spinophilin Mediates Striatal Facilitated Motor Function

2.1 Introduction

The striatum is one of the chief brain regions within the basal ganglia network which mediates motor control. Motor coordination, learning and performance are mediated in part by the dorsal striatum, more specifically the DLS (Balleine & O'Doherty, 2010; Durieux et al., 2009; Durieux et al., 2012; Stalnaker et al., 2012). In neurodegenerative disease states in which the striatum is greatly affected, such as PD and HD, dysregulated hypokinetic and hyperkinetic movements are observed (Bernheimer et al., 1973; McGregor & Nelson, 2019; Redgrave et al., 2010). In both cases, dopaminergic signaling dysfunction has been implicated as a critical determinant of the motor perturbations and strongly affect the DLS (Bernheimer et al., 1973; Burrus et al., 2020; Fernández-García et al., 2020; Kish et al., 1988). Thus, striatal MSN signaling is paramount for proper function within the DLS. Proper PP1 function is critical in maintaining appropriate MSN signaling. In addition, global loss of spinophilin, the major PP1 targeting protein at the postsynaptic density (Colbran et al., 1997), enhances the sedative effects of alpha2-adrenergic agonists (Lu et al., 2010). However, how loss of spinophilin impacts motor learning and performance using a rotarod motor-learning paradigm is unclear. Furthermore, the MSN subtype that may mediate spinophilin-dependent motor dysfunction within the striatum is also unknown. Here we demonstrate that whole body spinophilin knockout (KO) mice demonstrate motor learning and motor performance deficits during the rotarod task. In addition, using a novel conditional

spinophilin KO, we determined that loss of spinophilin in iMSNs, drives the motor impairments previously established in a rotarod task.

2.2 Materials and Methods

2.2.1 Animals

Adult (3-5.5 month) old whole-body spinophilin knock-out ((B6N(Cg)-*Ppp1r9b*$^{tm1.1(KOMP)Vlcg}$/J) (KO), wild-type (WT) C57BL/6, and adult (7-8 week old) Drd1-Cre (036916-UCD B6.FVB(Cg)-Tg(Drd1a-cre)FK150Gsat/Mmucd) (D1Cre); Adora2a-Cre (MMRRC strain 036158-UCD, B6.FVB(Cg)-Tg(Adora2a-cre)KG139Gsat/Mmucd) (A2Acre) (Gerfen et al., 2013; Gong et al., 2007); and mice expressing loxp sites around Exon 3 of spinophilin (spinophilin$^{Fl/Fl}$) were used in these studies. Spinophilin$^{Fl/Fl}$ were crossed with either D1- or A2A-Cre lines to selectively knock-out spinophilin in either dMSNs (Spino$^{\Delta dMSN}$) or iMSNs (Spino$^{\Delta iMSN}$), respectively. All mice were maintained on a C57BL/6 background. Male and female mice were used for all experiments; however, sex was not considered as a biological variable in the behavioral studies.

2.2.2 Generation and Validation of Conditional Spinophilin KO Mice

Conditional mice were generated at the university of Michigan Transgenic Animal Model Core. Embryonic stem cells (ESCs) containing a targeted insert with a beta-gal reporter and neomycin selection cassette encased by FRT sites as well as loxP sites flanking exon 3 of spinophilin were generated by the European Conditional Mouse Mutagenesis Program (EuCoMM) and validated using a long-range PCR. These ES cells were obtained from the European Mouse Mutant Cell Repository (EuMMCR) and directly sent to the University of Michigan where karyotyping was performed. Initial chimeras were

produced by microinjecting the ES cells into blastocysts obtained from the mating of C57BL/6-BrdCrHsd-Tyrc females with B6(cg)-Tyr<c2J>/J males. Chimeras were bred with C57Bl/6 mice and pups from these crosses were transferred to our laboratory. We then crossed these mice with a mouse line containing FlpO (Jackson laboratories B6.129S4-*Gt(ROSA)26Sor*$^{tm2(FLP*)Sor}$/J; Stock#012930). After crossing the newly generated mice with mice expressing FLP recombinase, only the loxP sites surrounding exon 3 of spinophilin remained. Functional validation of spinophilin$^{Fl/Fl}$ was performed via targeted genotyping in the laboratory and by selectively breeding spinophilin$^{Fl/Fl}$ or spinophilin$^{Fl/+}$ mice with mice that contained a tamoxifen-inducible Cre recombinase under the control of the chicken beta-actin promoter coupled with the cytomegalovirus (CMV) immediate early enhancer, (B6.Cg-Tg(CAG-cre/Esr1*)5Amc/J (CagCreER; Jackson #004682) which resulted in a conditional whole-body spinophilin KO (CagCreER$^{spinophilin\ floxed}$) (**Figure 9**). Immunoprecipitation of spinophilin from striatal tissue lysate along with immunoblotting was performed to validate the absence of spinophilin. The conditional spinophilin$^{Fl/Fl}$ mice were backcrossed to the C57BL/6 background for at least six generations before studies were performed in order to ensure pure C57Bl6/J background and to eliminate any off-target mutations from the blastocyst injection. We acknowledge and thank Wanda Filipiak & Galina Gavrilina for preparation of transgenic mice as well as the entire Transgenic Animal Model Core (in particular, Anna LaForest, Elizabeth Hughes, Corey Ziebell, and Dr. Thomas Saunders) and the University of Michigan's Biomedical Research Core Facilities for generation of the Spinophilin$^{Fl/Fl}$ mice.

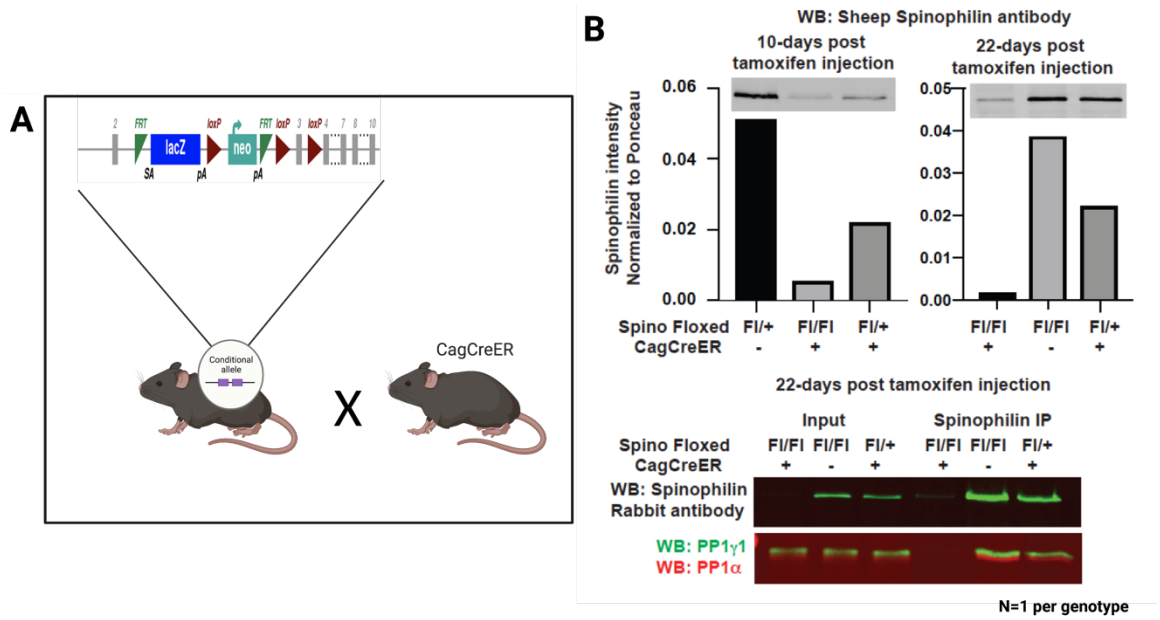

Figure 9: Preliminary Validation of Conditional Spinophilin Floxed Mouse. (A) Preliminary validation of spinophilin$^{Fl/Fl}$ was performed by crossing spinophilin$^{Fl/Fl}$ or spinophilin$^{Fl/+}$ mice with mice that contained a tamoxifen-inducible Cre recombinase under the control of the chicken beta-actin promoter coupled with the cytomegalovirus (CMV) immediate early enhancer (CagCreER) which resulted in a conditional whole-body spinophilin KO (CagCreER$^{spinophilin\ floxed}$). (B) Immunoprecipitation and immunoblotting of spinophilin from striatal tissue collected from tamoxifen or vehicle treated progeny of spinophilin floxed and CagCreER at 10- or 22-days post tamoxifen injections. Spinophilin in the striatum was shown to be reduced by at least 90-95%. In addition, after 22-days post injection PP1α and PP1γ1 was not detected in the spinophilin immunoprecipitates. Created in BioRender.com.

2.2.3 Animal Husbandry

Mice were provided food and water *ad libitum*. All sustenance was monitored daily by either laboratory or Science Animal Research Center (SARC) personnel and cages were changed weekly or bi-weekly. Animals were group housed with no more than five animals per cage and provided bedding and house nestlets. Cages and enrichment materials were changed weekly or bi-weekly. All animal experiments were approved by the School of Science Institutional Animal Care and Use Committee (SC270R, SC310R) and performed in accordance with the Guide for the Care and Use of Laboratory Animals and under the oversight of Indiana University-Purdue University, Indianapolis (IUPUI).

2.2.4 Animal Behavior: Rotarod

Mice were challenged for three consecutive days on an accelerating rotarod (4-40 rpm in 300 seconds) task, that is 3 cm in width (Rotamex-5, Columbus Instruments). Mice underwent three successive inter-session trials for three consecutive days, as previously described **(Figure 10**) (Edler et al., 2018). Conditional cell-specific spinophilin floxed mice were challenged using a modified version of the rotarod test, in which the test was extended to a total testing of 5 days (**Figure 11**) all other parameters did not change and was carried out as previously described (Edler et al., 2018). A series of photocell beams located above the rotating rod with a temporal resolution of 0.1 rpm (0.1 cm/sec) detects when the mouse is no longer on the rod. To circumvent any false fall recordings, any mouse that was able to grip the rod and rotate with the rod for two rotations or greater was also considered to have failed the trial and the data was not used.

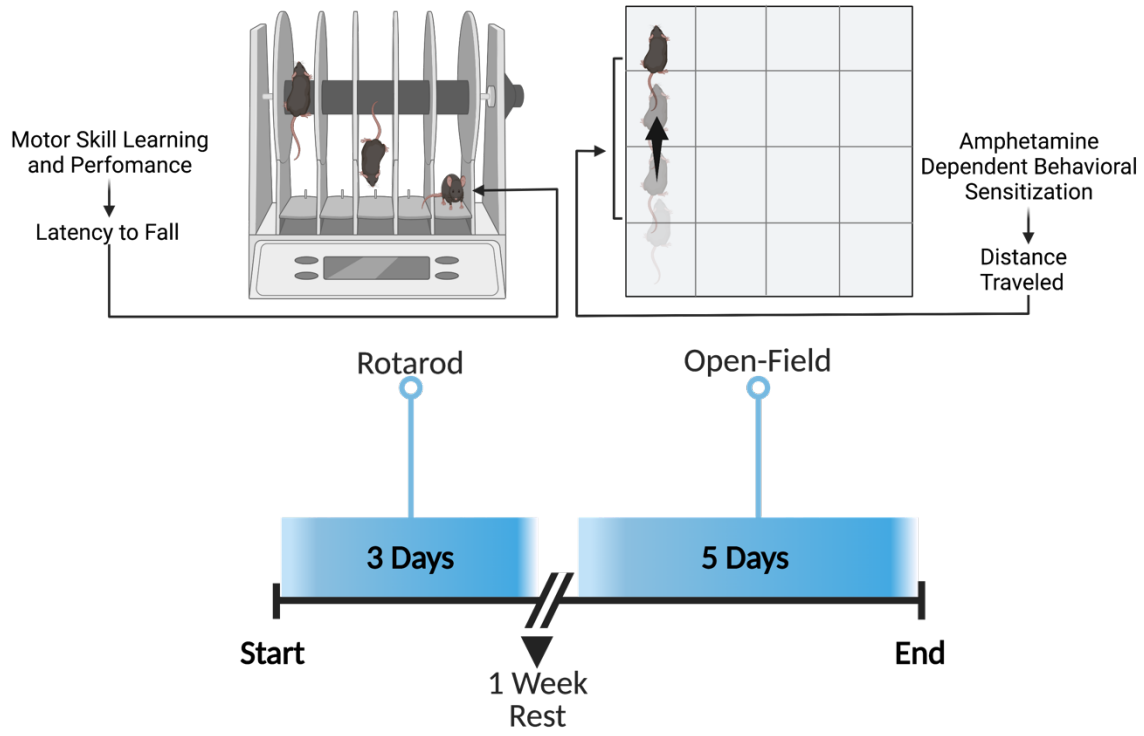

Figure 10: Striatal Motor Output Behavior Paradigm. Animals were challenged on an accelerating rotarod task as described by (Edler et al., 2018). Latency to fall was documented for each subject. Following training on the accelerating rotarod, subjects were given a rest period of one week and were then challenged with a sensitizing regimen of d-amphetamine as previously described (Morris et al., 2018) distance traveled was measured for 60-minutes and documented. Created with BioRender.com.

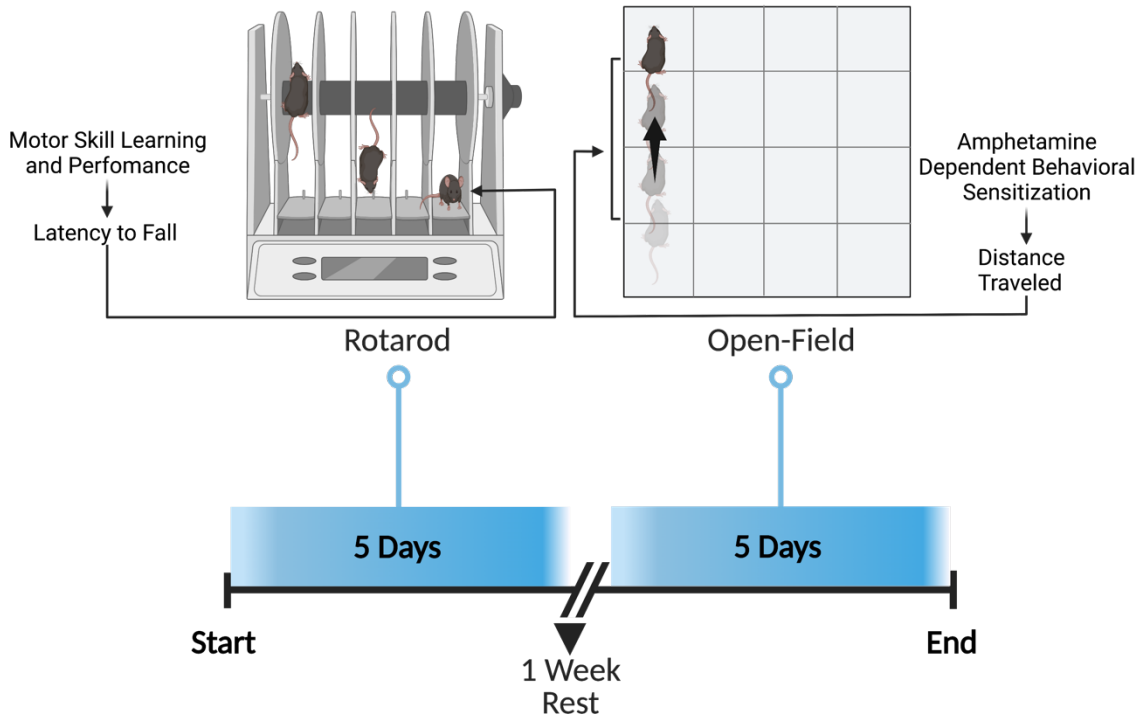

Figure 11: Modified Striatal Motor Output Behavior Paradigm. Animals were challenged on a modified accelerating rotarod task in which the number of days were extended to five; with other aspects of the protocol performed as previously described (Edler et al., 2018). Latency to fall was documented for each subject. Following training on the accelerating rotarod, subjects were given a rest period of one week and were then challenged with a sensitizing regimen of d-amphetamine previously described (Morris et al., 2018) distance traveled was measured for 60-minutes and documented using the Noldus Phenotyper Cages (30 cm X 30 cm X 30 cm) system. Created with BioRender.com.

Latency to fall was documented for each mouse, and a 120–180 s rest was given to all mice before the next trial.

2.2.5 Brain Tissue Lysis

Whole striatum was dissected from mice after live decapitation. Striatal tissue was homogenized and sonicated in 1.25 mLs of ice-cold, low ionic strength lysis buffer containing 2 mM Tris-HCl, 1 mM DTT, 2 mM EDTA, 1% Triton X-100, 1X protease inhibitor cocktail (Bimake, Houston, TX, USA), and phosphatase inhibitors (20 mM sodium fluoride, 20 mM sodium orthovanadate, 20 mM *β*-glycerophosphate, and 10 mM sodium pyrophosphate; Sigma-Aldrich or ThermoFisher Scientific), as previously described (Watkins et al., 2019, 2018).

2.2.6 Immunoprecipitation

Whole striatal lysates were immunoprecipitated with 4 µg of sheep spinophilin polyclonal antibody (ThermoFisher Scientific, PA5-48102) and were incubated at 4 °C with 800 µL (80%) of total striatal lysate on a rotating mixer overnight. The next day protein G magnetic beads (DynaBeads, ThermoFisher Scientific) were added, and the mixture was incubated for 2 h. Beads were washed three times by magnetic separation in an immunoprecipitation wash buffer (50 mM NaCl, 50 mM Tris-HCl pH 7.5, 0.5% (*v*/*v*) Triton X-100). Samples were then placed in -20 °C until immunoblotting.

2.2.7 Immunoblotting

Immunoprecipitates and inputs were separated by SDS-PAGE and blotted with the following primary antibodies: rabbit-spinophilin (Cell Signaling Technology, 14136S), sheep-spinophilin (Thermo; PA5-48102), goat-PP1γ1 (SantaCruz; sc-6108), mouse-PP1α (SantaCruz; sc-7482). Infrared secondary antibodies were used (Donkey anti goat, donkey anti rabbit, donkey anti mouse or donkey anti sheep conjugated to Alexa Fluor 690 or 780; ThermoFisher and Jackson Immunologicals) and imaged using Odyssey CLX and fluorescence intensity measurements were made using Image Studio (LI-COR Biosciences, Lincoln, NE). All primary antibodies were used at a (1:1000) dilution and secondaries at a (1:10,000) dilution. Immunoblotting was performed as previously described (Edler et al., 2018; Morris et al., 2018; Watkins et al., 2018).

2.2.8 Drug Administration

CagCreER$^{\text{spinophilin floxed}}$ mice were given 100μL tamoxifen concentrated at 24mg/mL via intraperitoneal (i.p) injections that was dissolved in sesame seed oil daily for five consecutive days. Mouse brains were harvested and processed for spinophilin deletion using immunoprecipitation and immunoblotting either 10 days or 22 days post-injection.

2.2.9 Statistics

Two-way analysis of variance (ANOVA) tests followed by Sidak's multiple comparison post-hoc tests were performed to determine if loss of spinophilin in either whole-body spinophilin KO or cell-specific spinophilin KO subjects had an effect on rotarod performance and learning when comparing across all trials. For experiments with

whole-body spinophilin KO, animals comparing the average of the three trials per day, a two-way ANOVA followed by a Sidak's multiple comparison post-hoc was performed. However, for the experiments with cell-specific conditional spinophilin floxed animals comparing the average of the three trials per day, a Mixed-effects analysis with Geisser-Greenhous correction was performed followed by a Dunnett's multiple comparison post-hoc test where all groups were compared to the control group. All values represent mean ± SEM. Statistical analyses and graphing were performed using Prism 9.3.1 (GraphPad, LaJolla, CA).

2.3 Results

Global Loss of Spinophilin Mediates Deficits in Rotarod Learning and Performance.

Loss of spinophilin has been shown to effect rotarod tests of sedation behavior (Lu et al., 2010; Wang et al., 2004). However, the basal effects of loss of spinophilin on a standard accelerating rotarod test was unclear. Here we have taken global spinophilin KO mice and wild-type (WT) controls through an accelerating rotarod test (**Methods and Materials**). Three trials per day for three consecutive days were performed, and the latency to fall was recorded (Edler et al., 2018). When comparing across all trials for a two-way ANOVA followed by a Sidak's multiple comparison post-hoc test revealed a significant trial effect ($F(8,90) = 2.093$; $p = 0.0444$) and a significant effect of genotype ($F(1,90) = 54.08$; $p < 0.0001$) with no interaction effect (**Figure 12B**). When comparing the average of the three trials per day a two-way ANOVA was performed followed by a Sidak's multiple comparison posthoc test using day and genotype as variables also

revealed a significant day effect (F(2,30) = 3.619; P = 0.0391) and a significant effect of genotype ($F(1,30) = 30.57$; $p < 0.0001$) with no interaction effect (**Figure 12C**). These data taken together suggest that global loss of spinophilin affects striatal mediated behaviors such as the rotarod task.

Loss of Spinophilin in iMSNs Mediates Deficits in Rotarod Performance and Learning.

Activation of striatal MSNs are differentially correlated with either increased or decreased movement, where increased activity in dMSNs is associated with an increase in movement and increased iMSN activation a decrease in movement (Lee et al., 2016). Although, our global spinophilin KO animals demonstrated deficits in rotarod tasks, it is unclear if striatal MSNs are mediating this response. We crossed the spinophilin floxed mouse with mice containing Cre recombinase under the control of a D1R or A2A receptor promoter, which will delete spinophilin specifically from either dMSNs (D1 Cre) or iMSNs (A2A Cre). We performed a more robust rotarod learning paradigm in which we extended the number of days of the test to five (**Figure 13A**). When comparing across trials (**Figure 13B**), a two-way ANOVA followed by a Sidak's multiple comparison post-hoc test revealed a significant trial effect ($F(14,675) = 20.47$; $p < 0.0001$). Furthermore, there was a significant effect of genotype ($F(2,675) = 34.56$; $p < 0.0001$) with no interaction effect (**Figure 13B**). When comparing the average of each three trials per day a Mixed-effects analysis with the Geisser-Greenhouse correction was performed on the average trial data shown (day and genotype as variables), followed by a Dunnett's multiple comparison post-hoc test where all groups were compared to the control group (**Figure 13C**).

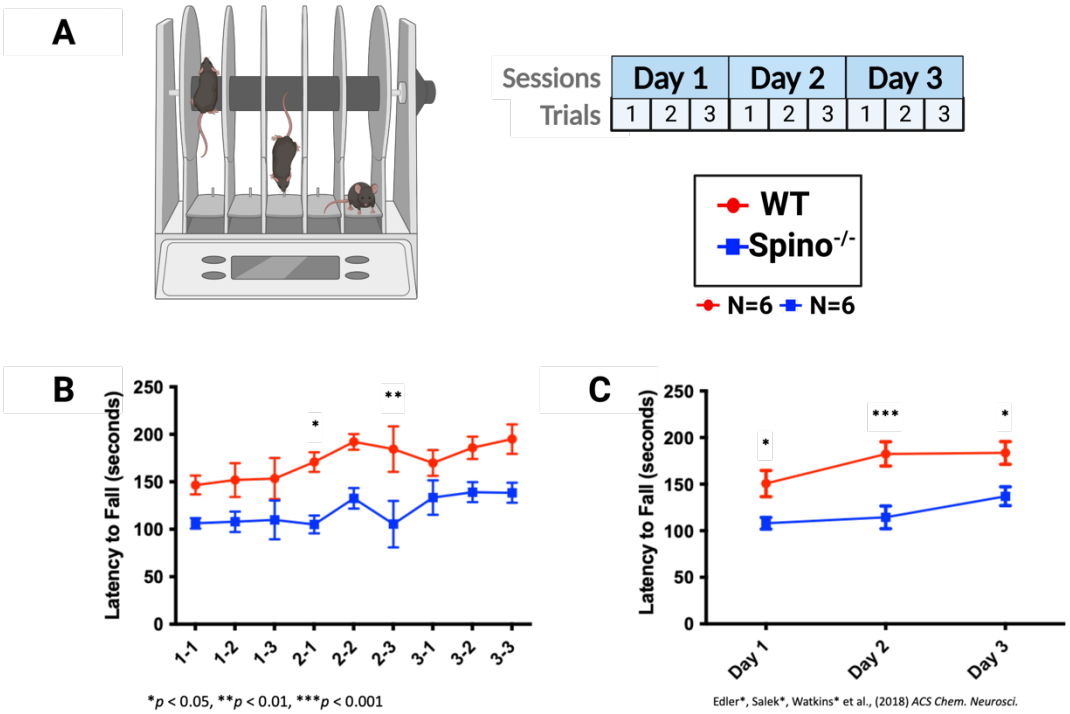

Figure 12: Rotarod Deficits in Spinophilin KO Mice. (A) Adult (3-5.5 month) old whole-body spinophilin KO ((B6N(Cg)-*Ppp1r9b*$^{tm1.1(KOMP)Vlcg}$/J) (red) and appropriate wild-type control mice (blue) were placed on an accelerating rotarod apparatus. Three trials per day for three consecutive days were performed, and the latency to fall was recorded (Edler et al., 2018). (B) All trials are shown: A two-way ANOVA followed by a Sidak's multiple comparison posthoc test was performed to compare across trials. A significant trial effect ($F(8,90) = 2.093$; $p = 0.0444$) and a significant effect of genotype ($F(1,90) = 54.08$; $p < 0.0001$) with no interaction effect. (C) The average of trials for each day. A two-way ANOVA was performed on the average trial data shown (day and genotype as variables). A significant day effect ($F(2,30) = 3.619$; $P = 0.0391$) and a significant effect of genotype ($F(1,30) = 30.57$; $p < 0.0001$) with no interaction effect. All values represent mean ± SEM. Modified/Adopted Figure from (Edler et al., 2018) Created with BioRender.com.

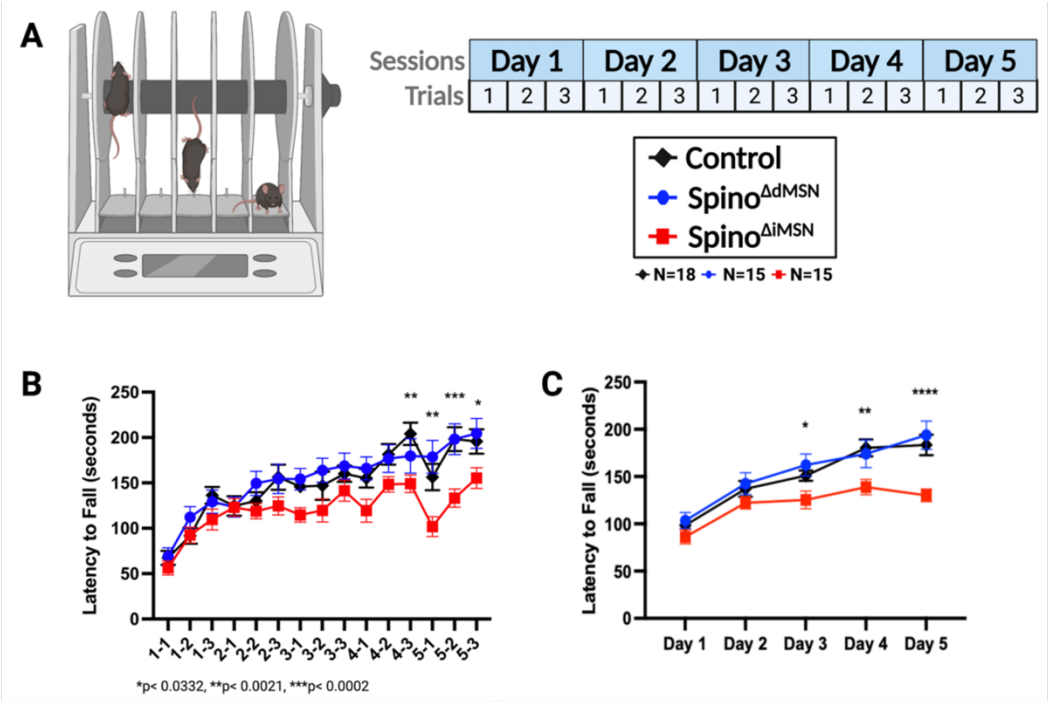

Figure 13: Rotarod Deficits in iMSN Spinophilin KO Mice. (A) Adult (7-8 week old) mice were placed on an accelerating rotarod apparatus. Three trials per day for five consecutive days were performed, and the latency to fall was recorded (modified methodology) (Edler et al., 2018). (B) All trials are shown- A two-way ANOVA followed by a Sidak's multiple comparison posthoc test was performed to compare across trials. There were significant trial ($F(14,675) = 20.47$; $p < 0.0001$) and genotype ($F(2,675) = 34.56$; $p < 0.0001$) effects with no interaction effect. (C) The average of trials for each day. A Mixed-effects analysis with the Geisser-Greenhouse correction was performed on the average trial data shown (day and genotype as variables), followed by a Dunnett's multiple comparison post-hoc test where all groups were compared to the control group. There was a significant day ($F(2.966,133.5) = 37.79$; $p<0.0001$, Geisser-Greenhouse's epsilon 0.7416) and genotype effect ($F(2,45) = 8.953$; $p < 0.0005$) with no interaction effect. All values represent mean ± SEM. Created with BioRender.com.

There was a significant day effect ($F(2.966,133.5) = 37.79$; $p<0.0001$, Geisser-Greenhouse's epsilon 0.7416) and significant genotype effect ($F(2,45) = 8.953$; $p < 0.0005$) with no interaction effect (**Figure 13C**). These data taken together suggest that loss of spinophilin in iMSNs is critical for rotarod motor learning and performance.

2.4 Discussion

Global Loss of Spinophilin Mediates Rotarod Behavioral Task

In our current understanding using the classical CST model (**Figure 4**), striatal projections innervate the globus pallidum which goes on to affect thalamic activity. It is possible that spinophilin mediates key molecular signaling events within the striatum through its ability to target PP1 to specific substrates, in response to environmental change within the MSN. In addition, spinophilin may also act as a PP1 inhibitor towards certain substrates and can thereby also reducing PP1-dependent dephosphorylation, thereby promoting increased substrate phosphorylation (Ragusa et al., 2010; Salek et al., 2019). Much like in animal models of PD, perturbations in dopaminergic and glutamatergic signaling have been shown to modulate rotarod performance (Ayton et al., 2013; Ogura et al., 2005). Specifically in 6-hydroxydopamine (6-OHDA) lesioned animals, a model of PD in which dopaminergic degeneration drives decreased dopaminergic tone within the striatum, deficits in rotarod performance and impairments to working memory are observed (Campos et al., 2013; Ogura et al., 2005). Furthermore, cAMP/PKA/DARPP-32 signaling mediates, in part, motor skill learning and performance and this pathway can be modulated via dopamine signaling (Qian et al., 2015).

Researchers found that inhibiting DARPP-32 activity at Thr34 diminished motor learning and skilled performance (Qian et al., 2015). In addition, PP1 has been shown to modulate glutamatergic signaling by its colocalization with spinophilin and AMPARs (Yan et al., 1999). The phosphorylation state of specific serine subunits on GluA1 mediate receptor trafficking, conductance, and expression (Carvalho et al., 2000; Jenkins & Traynelis, 2012; Wang et al., 2005). These data taken together suggest that it is likely loss of spinophilin disrupts PP1 regulation of several synaptic proteins. The aberrant changes in phosphorylation states can drive deficits in motor skill learning and performance (Qian et al., 2015). In addition, the rotarod motor learning has distinct phases (skill learning associated with the early phase and performance associated with the late phase) (Costa et al., 2004; Luft & Buitrago, 2005). Loss of spinophilin may dysregulate AMPA/NMDA ratios via PP1 dysregulation, which are critical in striatal-mediated motor skill learning and performance (Hawes et al., 2015; Rothwell et al., 2015; Shan et al., 2014). The rotarod deficits demonstrated with the global spinophilin KO indicate that spinophilin plays a role; however, how spinophilin functions cell-specifically will aid in unmasking specific mechanisms that mediate the motor skill learning and performance deficits demonstrated in the rotarod task.

Loss of Spinophilin within iMSNs Mediates Some Rotarod Performance Perturbations

Global loss of spinophilin mediated rotarod deficits (**Figure 12B-C**). However, using a novel conditional spinophilin floxed mouse, we were able to demonstrate that loss of spinophilin in iMSNs also produce deficits in the rotarod task (Figure **13B-C**). The indirect pathway in the CST model (Figure **4**) is associated with decreases in movement

(Calabresi et al., 2014; Hauber, 1998; Lee et al., 2016; Wang et al., 2015). In addition, recently Augustin et al. revealed that loss of D2Rs within iMSNs drive deficits in motor skill learning, more specifically rotarod tasks (Augustin et al., 2020). It is possible that disruptions in spinophilin and PP1 interactions specifically impair iMSN signaling while dMSNs may have a compensatory mechanism allowing for proper signaling during a rotarod challenge. Furthermore, spinophilin directly binds D2Rs at the third intercellular loop (IC3) (Smith et al., 1999). Interestingly, D2Rs also have a phosphorylation-dependent mechanism that mediates receptor internalization on several serine/threonine sites at the second intracellular loop (IC2) and a threonine site on IC3 (Cho et al., 2010). Thus, loss of spinophilin in iMSNs may increase the internalization of D2Rs, which are expressed in iMSNs, but not dMSNs. Furthermore, striatal cAMP signaling is limited in iMSNs by D2Rs (Augustin et al., 2014; Augustin et al., 2020). The cAMP/PKA signaling pathway increases phosphorylation of DARPP-32 at Thr34, inhibiting PP1 actions (Greengard et al., 1999; Yger & Girault, 2011). Thus, loss of spinophilin in iMSNs could drive D2R internalization and further limit PP1 regulatory actions indirectly, by increasing cAMP/PKA signaling. Furthermore, typical activation of D2Rs decrease AMPA receptor currents (Cepeda et al., 1993; Hernández-Echeagaray et al., 2004). Loss of spinophilin in iMSNs may be compounding AMPA receptor current dysregulation causing increased glutamatergic synaptic transmission increasing iMSN function which is correlated with decreases in movement (Lee et al., 2016). In addition, late phase of the rotarod task has the greatest deficit, suggesting that loss of spinophilin in the DLS is critical to properly perform the tasks. Indeed, D2Rs are critical for habitual actions in the DLS (Shan et al., 2015). However, loss of spinophilin in iMSNs did not fully recapitulate

what was observed in the global spinophilin KO animals. In the global KOs there was a significant difference in performance of the task starting on day 1 (**Figure 12C**). A significant difference in the iMSN spinophilin KO mice was not observed until day 3 (**Figure 13C**). Due to no significant deficits in the dMSN spinophilin KO animals, the reason for the basal deficits in the global spinophilin KOs may be due to perturbations in function in other brain regions. For instance, using multimodal MRI imaging, the hippocampus and frontal cortex show an increase in volume in mice that have been trained on the rotarod task, demonstrating these brain regions to play a critical role in motor skill learning tasks like the rotarod (Scholz et al., 2015). In addition, motor dexterity and forelimb reaching is critical to motor skill learning and task performance such as the rotarod, and cerebello-thalamic circuitry is critical in accomplishing such task (Sakayori et al., 2019). Indeed, spinophilin is expressed in the hippocampus, frontal cortex, cerebellum, and the thalamus (Allen et al., 1997; E. C. Muly et al., 2004) and additional studies are warranted to detail spinophilin function within cell types in these brain regions.

2.5 Conclusion

Global loss of spinophilin drives performance deficits in the rotarod task as evidenced when mice were challenged to perform a rotarod motor learning task. When spinophilin was knocked out cell type-specifically in either D1-containing MSNs (dMSNs) or A2A-containing MSNs (iMSNs), rotarod deficits were only observed in iMSN spinophilin KOs. These data together suggest that spinophilin's regulatory actions and protein-protein interactions are critical for appropriate striatal-mediated behaviors. In addition, spinophilin function within the dorsal striatum aids in facilitating striatal-mediated motor actions. Furthermore, spinophilin appears to mediate the rotarod task in a cell-specific manner.

Chapter III: Spinophilin Mediates Amphetamine-Induced Striatal Motor Adaptations

3.1 Introduction

Psychostimulant drugs of abuse, such as methamphetamine, amphetamine, and cocaine, are associated with dopamine (DA) dysfunction, improper cortico-striatal synaptic transmission, and other neuronal perturbations, such as enhanced DA receptor sensitization that may lead to psychostimulant abuse and the transition from abuse to addiction (Burke & Miczek, 2014; Gerdeman et al., 2003; Holder et al., 2015; Sanchez-Ramos, 2015; Yager et al., 2015). Repeated psychostimulant exposure over time gives rise to psychostimulant sensitization, predominantly via increased responsivity to striatal dopaminergic transmission (Peter W. Kalivas & Jane Stewart, 1991; Robinson & Becker, 1986; Steketee & Kalivas, 2011). Psychostimulant-induced sensitization can be observed behaviorally and the striatum is a critical brain region for mediating behaviors associated with psychostimulant sensitization and addiction (Hooks et al., 1992). Behavioral sensitization can be characterized as the process by which there is augmented motor responses that are in concert with intermittent psychostimulant administration (**Figure 14**) (Peter W. Kalivas & Jane Stewart, 1991; Robinson & Becker, 1986; Steketee & Kalivas, 2011). It is important to note that psychostimulant sensitization is not a direct correlate to psychostimulant addiction; however, both phenomena may share conserved overlapping neural mechanisms, which makes elucidating the mechanisms by which psychostimulant sensitization is mediated in the striatum promising for a better understanding of the disease (Vezina, 2007). Due to the rewarding and reinforcing effects

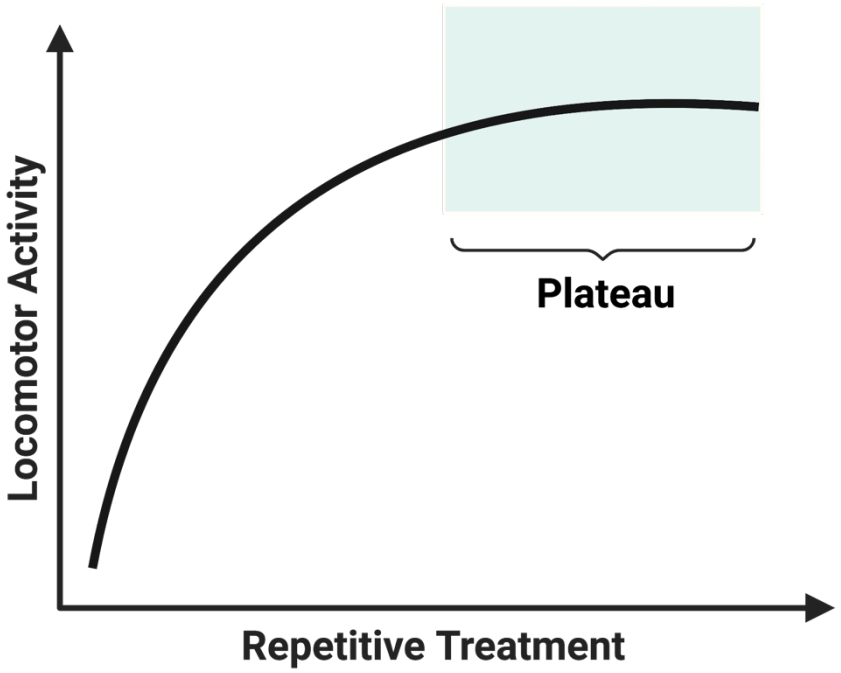

Figure 14: Psychostimulant-Induced Behavioral Sensitization. Simple schema of psychostimulant-induced behavioral sensitization. Repeated treatment of psychostimulants can cause incremental increases in locomotor activity. Eventually, the increase in locomotion plateaus. Created with BioRender.com.

of psychostimulants, much of the literature has focused on ventral striatal function in the context of psychostimulant addiction; however, emerging research suggests that the dorsal striatum plays an essential role in psychostimulant-induced behavioral responses, which is a major contributor to relapse (Volkow et al., 2006).

In addition to behavioral responses, molecular neuroadaptations in MSNs are thought to mediate psychostimulant sensitization, in that there are distinct interactions involving dopaminergic and glutamatergic transmission via kinase signaling pathways that give rise to the behavioral response (**Figure 15**) (Allichon et al., 2021; Bateup et al., 2010; Lobo & Nestler, 2011; Salery et al., 2020; Valjent et al., 2006). Furthermore, a sensitizing regimen of amphetamine was shown to increase striatal spinophilin expression (Boikess & Marshall, 2008; Boikess et al., 2010). Moreover, global loss of spinophilin enhances cocaine-induced behavioral responses (Allen et al., 2006). It is unclear how loss of spinophilin affects amphetamine-induced locomotor sensitization. Here we demonstrate that global loss of spinophilin abrogates amphetamine-induced locomotor sensitization and that loss of spinophilin from either dMSNs or iMSNs alone does not recapitulate what is observed in the global spinophilin KOs.

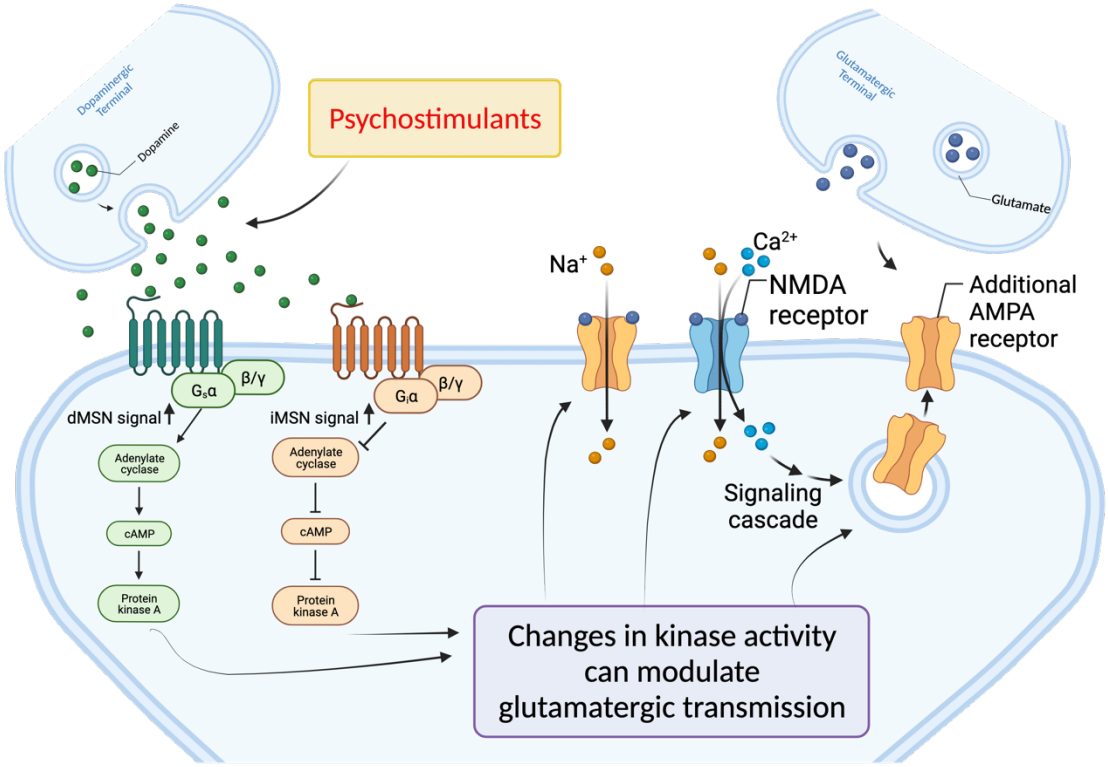

Figure 15: Psychostimulants Increase Dopaminergic Signaling and Modulate Glutamatergic Signaling. Psychostimulants increases the neuromodulator DA within the synaptic cleft driving sustained dopaminergic transmission. Sustained signaling from DARs drives changes in kinase activity which go on to modulate glutamatergic transmission via changes in conductance, and trafficking. Created with BioRender.com.

3.2 Materials and Methods

3.2.1 Animals

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3.2.2 Generation and Validation of Conditional Spinophilin KO Mice

Conditional mice were generated at the university of Michigan Transgenic Animal Model Core. Embryonic stem cells (ESCs) containing a targeted insert with a beta-gal reporter and neomycin selection cassette encased by FRT sites as well as loxP sites flanking exon 3 of spinophilin were generated by the European Conditional Mouse Mutagenesis Program (EuCoMM) and validated using a long-range PCR. These ES cells were obtained from the European Mouse Mutant Cell Repository (EuMMCR) and directly sent to the University of Michigan where karyotyping was performed. Initial

chimeras were produced by microinjecting the ES cells into blastocysts obtained from the mating of C57BL/6-BrdCrHsd-Tyrc females with B6(cg)-Tyr<c2J>/J males. Chimeras were bread with C57Bl/6 mice and pups from these crosses were transferred to our laboratory. We then crossed these mice with a mouse line containing FlpO (Jackson laboratories B6.129S4-*Gt(ROSA)26Sor*$^{tm2(FLP*)Sor}$/J; Stock#012930). After crossing the newly generated mice with mice expressing FLP recombinase, only the loxP sites surrounding exon 3 of spinophilin remained. Functional validation of spinophilin$^{Fl/Fl}$ was performed via targeted genotyping in the laboratory and by selectively breeding spinophilin$^{Fl/Fl}$ or spinophilin$^{Fl/+}$ mice with mice that contained a tamoxifen-inducible Cre recombinase under the control of the chicken beta-actin promoter coupled with the cytomegalovirus (CMV) immediate early enhancer, (B6.Cg-Tg(CAG-cre/Esr1*)5Amc/J (CagCreER; Jackson #004682) which resulted in a conditional whole-body spinophilin KO (CagCreER$^{spinophilin\ floxed}$) (**Figure 9**). Immunoprecipitation of spinophilin from striatal tissue lysate along with immunoblotting was performed to validate the absence of spinophilin. The conditional spinophilin$^{Fl/Fl}$ mice were backcrossed to the C57BL/6 background for at least six generations before studies were performed in order to ensure pure C57Bl6/J background and to eliminate any off-target mutations from the blastocyst injection. We acknowledge and thank Wanda Filipiak & Galina Gavrilina for preparation of transgenic mice as well as the entire Transgenic Animal Model Core (in particular, Anna LaForest, Elizabeth Hughes, Corey Ziebell, and Dr. Thomas Saunders) and the University of Michigan's Biomedical Research Core Facilities for generation of the Spinophilin$^{Fl/Fl}$ mice.

3.2.3 Animal Husbandry

Mice were provided food and water ad libitum. All sustenance was monitored daily by either laboratory or Science Animal Research Center (SARC) personnel and was changed weekly or bi-weekly. Animals were group housed with no more than five animals per cage and provided bedding and house nestlets. Cages and enrichment materials were changed weekly or bi-weekly. All animal experiments were approved by the School of Science Institutional Animal Care and Use Committee (SC270R, SC310R) and performed in accordance with the Guide for the Care and Use of Laboratory Animals and under the oversight of Indiana University-Purdue University, Indianapolis (IUPUI).

3.2.4 Animal Behavior: Amphetamine Induced Behavioral Sensitization

A sensitizing regimen of d-amphetamine (3 mg/kg) were administered every 24 hours for five days, similar to previously described (Morris et al., 2018). Whole-body spinophilin KO and WT mice were tested using the VersaMax Animal Activity Monitoring System (Accuscan Instruments Inc., Columbus, OH). The 40 × 40 cm test chamber was equipped with evenly spaced intersecting photocell beams along the walls of the test chamber. Interruptions in intersecting photocell beams detected locomotor activity. Sound attenuating test chambers were situated in a 53 cm across x 58 cm deep x 40 cm high chamber with a house light and fan for ventilation and background noise as previously described in (Boehm et al., 2008; Morris et al., 2018). While D1-Cre and A2A-Cre; spinophilin$^{Fl/Fl}$, spino$^{\Delta dMSN}$ and Spino$^{\Delta iMSN}$ were tested using individually housed Noldus Phenotyper Cages (30 cm X 30 cm X 30 cm) that were concealed to prevent mice from visualizing each other. Briefly, mice were given an intraperitoneal

(i.p.) injection of 3.0 mg/kg of d-amphetamine or saline vehicle (10 mL/kg). d-Amphetamine was administered once every 24 hours for five consecutive days. Immediately after injection, mice were placed in either the VersaMax apparatus (Figure **10**) or the Noldus Phenotyper Cages (Figure **11**). The VersaMax apparatus is interfaced with a Dell computer and software that is programed to track consecutive photocell beam interruptions that could be translated into distance traveled (cm). Data was collected in 1-minute intervals for 60 minutes. The Noldus Phenotyper Cages are interfaced with a stand-alone Dell computer in which visualization software (EthoVision XT) is used to track locomotion (distance traveled). Immediately upon placing animals in the phenotyper cages the software calibrated the subject's body to a single identifier localized to detect the center of the mouse's body. Data was collected in 1-second intervals for 60 minutes.

3.2.5 Drugs

d-amphetamine hemisulfate salt was purchased from Sigma-Aldrich, (St. Louis, MO, USA)

3.2.6 Statistics

Two-way analysis of variance (ANOVA) tests followed by either a Tukey or Sidak post-hoc test was performed to determine if loss of spinophilin in whole-body spinophilin KO would affect amphetamine mediated behavioral sensitization. Three-way ANOVA tests with repeated measures were performed to determine if there were significant affects to amphetamine mediated behavioral sensitization with loss of

spinophilin in either dMSNs or iMSNs. In, addition Three-way ANOVA tests with repeated measures were performed on A2A- and D1-Cre controls. All values represent mean ± SEM. Statistical analyses and graphing were performed using Prism 9.3.1 (GraphPad, LaJolla, CA).

3.3 Results

Global Loss of Spinophilin Abrogates Amphetamine-induced Behavioral Sensitization

Repeated amphetamine administration is known to induce locomotor sensitization in concert with myriad protein expression changes within the striatum (Boikess & Marshall, 2008; Boikess et al., 2010; Schmidt et al., 2010). Here we administered a sensitizing amphetamine regimen of amphetamine to either WT or spinophilin KO animals and measured the total distance travelled for 1 hour (Figure **16A**). A Two-Way ANOVA shows a significant genotype effect ($F(1,4) = 8.827$; $P = 0.0411$), and a significant interaction (Genotype and Day) effect ($F(4,16) = 18.80$; $P<0.0001$) (Figure **16B**). A Tukey post-hoc test comparing the means within each genotype across days shows a significant increase in locomotion in WT amphetamine treated animals at days 2 −5 compared to day 1 (***** symbols) (Figure **16B**). In contrast, there is no difference in locomotor activity in the spinophilin KO mice at days 2–4, and a significant decrease at day 5 (# symbol), compared to day 1(Figure **16B**). Furthermore, a Sidak post-hoc test across genotypes of amphetamine-treated animals at each day revealed significant differences in locomotion between WT and KO animals at days 3–5 (* symbols) (**Figure 16B**). Together the data suggest that loss of spinophilin attenuates the behavioral sensitization associated with repeated amphetamine exposure.

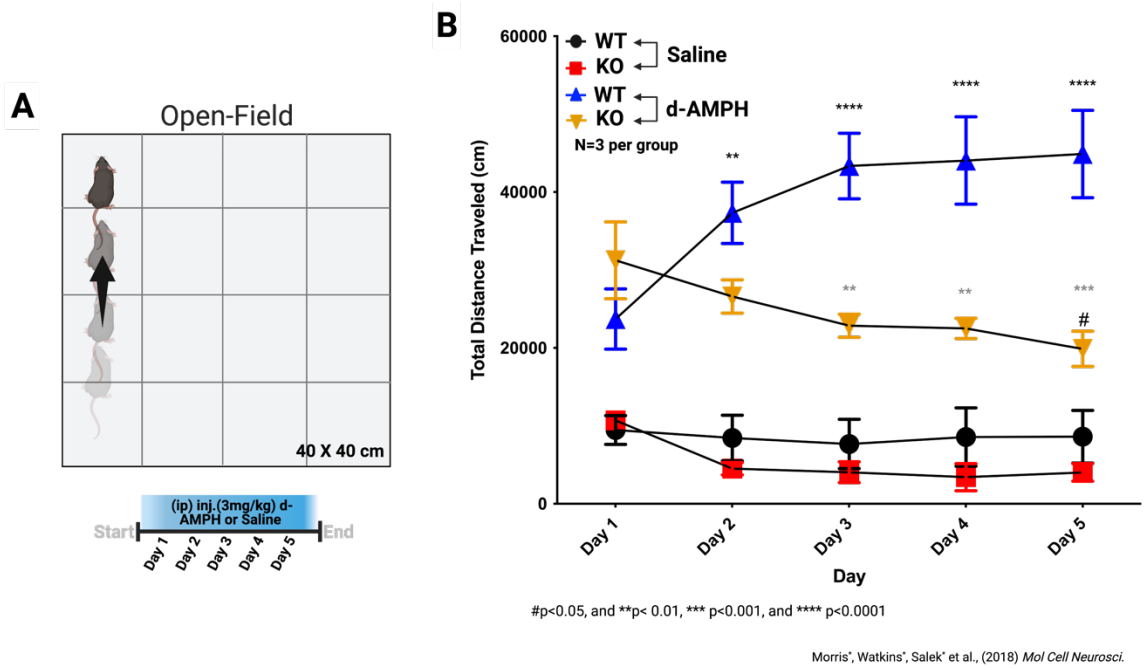

Figure 16: Attenuation of Amphetamine-dependent Behavioral Sensitization in Spinophilin KO Mice. (A) Adult (3-5.5 month) old whole-body spinophilin KO ((B6N(Cg)-*Ppp1r9b*$^{tm1.1(KOMP)Vlcg}$/J) and appropriate wild-type control mice were given an intraperitoneal (i.p.) injection of 3.0 mg/kg of d-amphetamine (Sigma) or the saline volume equivalent to the control groups once every 24 hours for five consecutive days. Immediately after each i.p. injection, mice were placed in a VersaMax Animal Activity Monitoring System (40 cm x 40 cm x 40 cm) sound attenuating test chambers. Distance travelled was collected for 1 hour. (B) Two-Way ANOVA shows a significant genotype effect ($F(1,4) = 8.827$; $P = 0.0411$), and a significant interaction (Genotype and Day) effect ($F(4,16) = 18.80$; $P<0.0001$). A Tukey post-hoc test comparing the means within each genotype across days shows a significant increase in locomotion in WT amphetamine treated animals at days 2 −5 compared to day 1 (* symbols). In contrast, there is no difference in locomotor activity in the spinophilin KO mice at days 2–4, and a significant decrease at day 5(# symbol), compared to day 1. Furthermore, a Sidak post-

hoc test across genotypes of amphetamine-treated animals at each day revealed significant differences in locomotion between WT and KO animals at days 3–5 (* symbols). All values represent mean ± SEM. Modified/Adopted Figure from (Morris et al., 2018) Created with BioRender.com.

Amphetamine-induced Behavioral Sensitization is Intact in Animals with MSN Cell Type-specific Loss of Spinophilin

Global loss of spinophilin attenuates psychostimulant-induced locomotor sensitization (**Figure 16**) (Areal et al., 2019; Morris et al., 2018). Loss of spinophilin in iMSNs displayed striatal mediated motor-skill learning and performance deficits (**Figure 13**). However, if cell-specific loss of striatal spinophilin mediates the ablation of amphetamine-induced locomotor sensitization exhibited in the global spinophilin KOs is unclear. We treated control (D1-Cre or A2A-Cre lines), spinophilin$^{Fl/Fl}$, spino$^{\Delta dMSN}$, or spino$^{\Delta iMSN}$ mice with either saline or a sensitizing regimen of amphetamine and found that locomotor sensitization was intact in all groups (**Figures 17-20**). These data taken together suggest spinophilin signaling in at least one MSN subtype is sufficient to achieve locomotor sensitization with a sensitizing regimen of amphetamine.

3.4 Discussion

Spinophilin Whole Body KO Mice do not Undergo Amphetamine-induced Sensitization

Here we demonstrate for the first time that amphetamine-induced behavioral sensitization is ablated in whole-body spinophilin KO animals (Morris et al., 2018). In fact, a year later Areal et al. showed that cocaine-induced behavioral sensitization was also lost in whole-body spinophilin KOs (Areal et al., 2019). Thus, regardless of psychostimulant type, spinophilin plays a critical role in behavioral sensitization. Mechanisms that mediate psychostimulant-induced locomotor sensitization are not fully

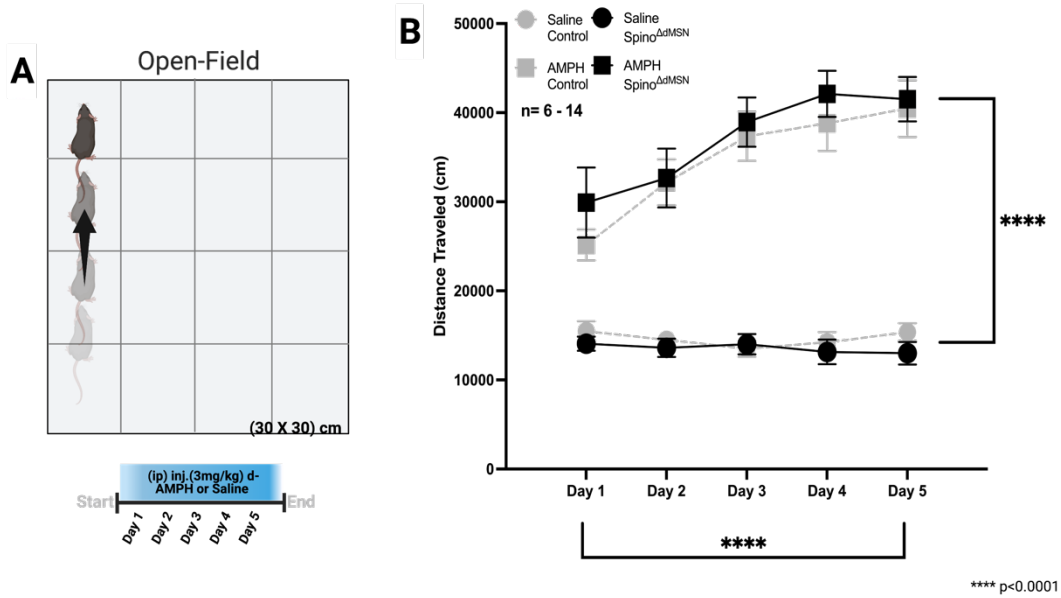

Figure 17: Intact Amphetamine-dependent Behavioral Sensitization in dMSN Spinophilin KO Mice. (A) 8-9 week old male and female spino$^{\Delta dMSN}$ along with appropriate control mice underwent an amphetamine-induced behavioral sensitization regimen, as previously described (Morris et al., 2018). Immediately after each injection, mice were placed in a Noldus Phenotyper Cage (30 cm x 30 cm x 30 cm). Each subject's distance travelled was recorded for 1 hour. (B) A three-way ANOVA with repeated measures were performed to determine effects that loss of spinophilin in dMSNs have on amphetamine-induced locomotor sensitization. Analysis found a significant day ($F(4, 144) = 29.73$, $p<0.0001$), and treatment effect ($F(1, 36) = 81.49$, $p<0.0001$), but no day x genotype ($F(4, 144) = 0.9587$, $p=0.43$) or treatment x genotype ($F(1, 36) = 0.4640$, $p=0.50$) interaction. All values represent mean ± SEM. Created with BioRender.com.

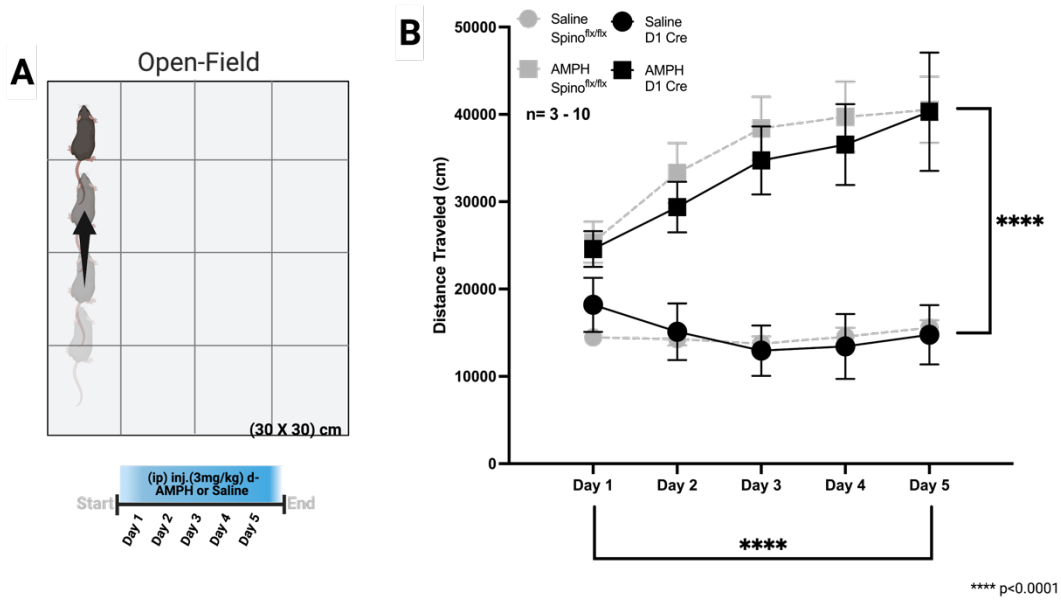

Figure 18: Intact Amphetamine-dependent Behavioral Sensitization in D1Cre Controls. (A) 8-9 week old male and female D1-Cre mice along with appropriate control mice, spinophilin$^{Fl/FL}$, underwent an amphetamine-induced behavioral sensitization regimen, as previously described (Morris et al., 2018). Immediately after each injection, mice were placed in a Noldus Phenotyper Cage (30 cm x 30 cm x 30 cm). Each subject's distance travelled was recorded for 1 hour. (C) A three-way ANOVA analysis determining the effects of D1 Cre only on amphetamine-induced locomotor sensitization showed a significant day ($F(4, 84) = 14.97$, $p<0.0001$) and treatment ($F(1, 21) = 31.63$, $p<0.0001$) effect on distance traveled, but no day x genotype ($F(4, 84) = 1.151$, $p=0.33$) or treatment x genotype ($F(1, 21) = 0.1531$, $p=0.69$) effect. All values represent mean ± SEM. Created with BioRender.com.

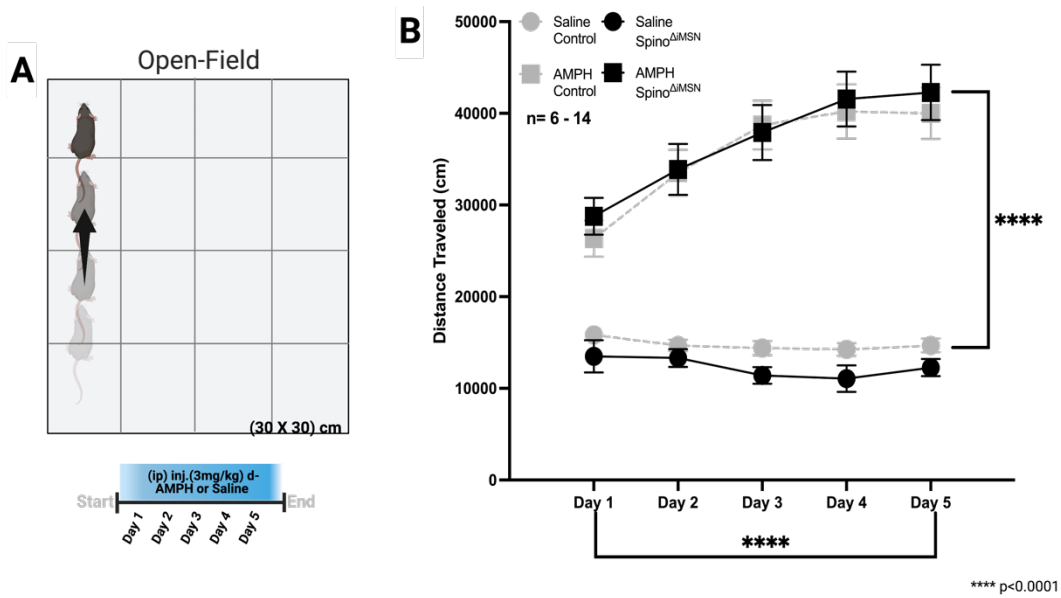

Figure 19: Intact Amphetamine-dependent Behavioral Sensitization in iMSN Spinophilin KO Mice. (A) 8-9 week old male and female spino$^{\Delta iMSN}$ along with appropriate control mice underwent an amphetamine-induced behavioral sensitization regimen, as previously described (Morris et al., 2018). Immediately after each injection, mice were placed in a Noldus Phenotyper Cage (30 cm x 30 cm x 30 cm). Each subject's distance travelled was recorded for 1 hour. (B) A three-way ANOVA with repeated measures were performed to determine effects that loss of spinophilin in iMSNs have on amphetamine-induced locomotor sensitization. Analysis found a significant day ($F(3.009, 117.4) = 28.46$, $p<0.0001$), and treatment effect ($F(1, 39) = 116.5$, $p<0.0001$), but no day x genotype ($F(4, 156) = 0.6937$, $p=0.59$) or treatment x genotype ($F(1, 39) = 0.7199$, $p=0.40$) interaction. All values represent mean ± SEM. Created with BioRender.com.

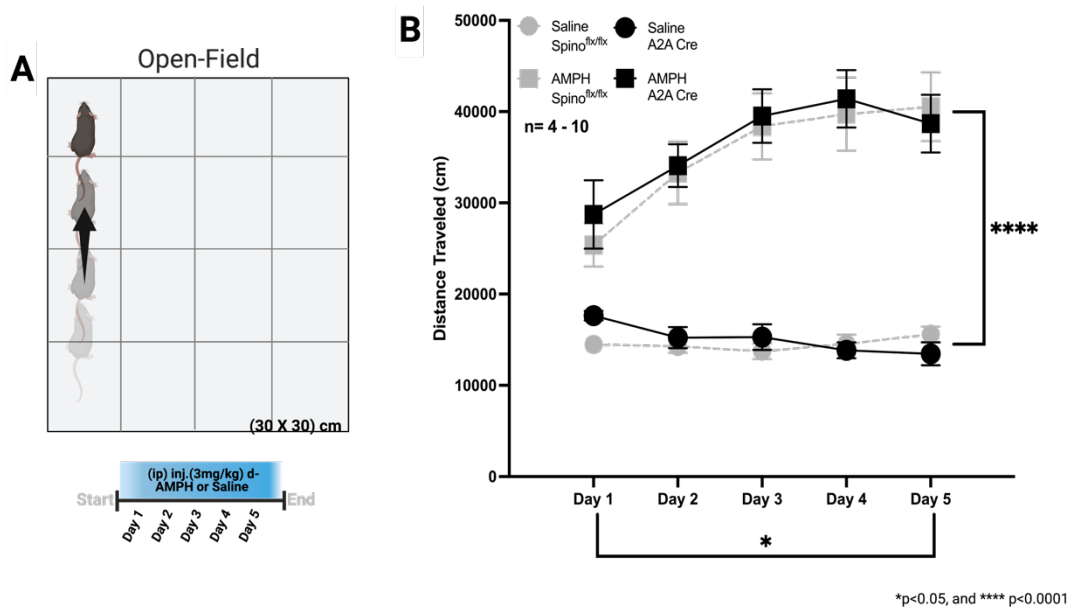

Figure 20: Intact Amphetamine-dependent Behavioral Sensitization in A2A Cre Controls. (A) 8-9 week old male and female A2A-Cre along with appropriate control mice spinophilin$^{FL/FL}$ underwent an amphetamine-induced behavioral sensitization regimen, as previously described (Morris et al., 2018). Immediately after each injection, mice were placed in a Noldus Phenotyper Cage (30 cm x 30 cm x 30 cm). Each subject's distance travelled was recorded for 1 hour. (B) A three-way ANOVA analysis determining the effects of A2ACre only on amphetamine-induced locomotor sensitization showed a significant day ($F(4, 120) = 2.859$, $p=0.026$) and treatment ($F(4, 120) = 260.4$, $p<0.0001$) effect on distance traveled, but no day x genotype ($F(4, 120) =0.4149$, $p=0.79$) or treatment x genotype ($F(1, 120) = 0.02898$, $p=0.86$) effect. All values represent mean ± SEM. Created with BioRender.com.

understood. However, myriad research suggests that striatal signaling dysregulation through dopaminergic and glutamatergic signaling are major contributors (Dela Peña et al., 2015; Kalivas, 2007; Kalivas et al., 2009; Peter W. Kalivas & Jane Stewart, 1991). PP1, the major spinophilin targeting protein, is a regulator of MSN synaptic signaling, and has been shown to regulate glutamatergic synaptic transmission and dendritic spine modulation, both of which are dysregulated with psychostimulant administration (Allichon et al., 2021; Hu et al., 2008; Jedynak et al., 2016; Jedynak et al., 2007; Munton et al., 2004). Furthermore, spinophilin anchors PP1 to glutamatergic targets, such as NMDARs and AMPARs, known mediators of glutamatergic synaptic transmission and dendritic spine dynamics via its PDZ binding domain (Feng et al., 2000; Salek et al., 2019; Sarrouilhe et al., 2006; Yan et al., 1999). The initial acute response to amphetamine on day 1 was normal when compared to the WT controls (**Figure 16**), suggesting that basal MSN responsivity to psychostimulants was unaltered. However, amphetamine administration gives rise to increased spinophilin expression (Boikess & Marshall, 2008; Boikess et al., 2010). Indeed, we began to see deficits starting at day 2 of amphetamine administration (**Figure 16**). These data together suggest that with repeated administration of psychostimulants, appropriate PP1-mediated signaling could be disrupted due to the absence of spinophilin giving rise to aberrant glutamatergic signaling. In addition, sensitization is coupled with plastic changes in myriad post-synaptic proteins; however, loss of spinophilin may prevent the molecular neuroadaptations needed to permit sensitization.

Loss of Spinophilin in dMSNs or iMSNs is not sufficient to abrogate amphetamine-induced sensitization

Repeated psychostimulant exposure inducing behavioral-sensitization has been associated with increased dMSN signaling (D'Souza, 2015), while disruptions in iMSN signaling in the DLS ablates psychostimulant-induced locomotor responses (Durieux et al., 2009; Durieux et al., 2012). In addition, our previous findings suggest that loss of spinophilin in iMSNs can mediate, in part, striatal-mediated motor behaviors (**Figure 13**). It is generally accepted that kinase and phosphatase signaling is critical for the proper integration of dopaminergic and glutamatergic transmission in MSNs; for example, the phosphorylation status of DARPP-32 can either inhibit phosphatase signaling or increase kinase signaling (**Figure 7**) (Fernandez et al., 2006; Yger & Girault, 2011). In addition, increases in dopaminergic signaling via psychostimulants can tip the balance of DARPP-32 phosphorylation cell type-specifically favoring the increase of PKA actions (Bateup et al., 2008). Indeed, psychostimulants drive bidirectional changes in the phosphorylation status of DARPP-32 at Thr34 (Bateup et al., 2008). Taken together the data suggest psychostimulants induce cell type-specific changes in the striatum, driving MSN signaling imbalances that give rise to neuroadaptations that permit psychostimulant sensitization. However, our data suggest that if these changes are occurring, intact spinophilin signaling in at least one MSN subtype is sufficient to achieve locomotor sensitization with a sensitizing regimen of amphetamine (**Figure 17-20**). Interestingly, given that the global spinophilin KOs did not undergo amphetamine sensitization and spinophilin loss from either MSN cell-type did not reproduce the global KO data, a higher order organization of circuitry or cell-type may be responsible. Certainly, although

interneurons account for less than ~5% of the neurons within the striatum, they play a major role in organizing MSN output (Gritton et al., 2019; Tepper & Koós, 2016). Specifically, parvalbumin interneurons mediate motor actions by fine-tuning striatal MSN output during motor execution, while cholinergic interneurons consolidate and organize MSN signaling output that signal the termination of a motor action (Gritton et al., 2019). Intriguingly, loss of D2Rs in cholinergic interneurons nullified psychostimulant-induced behavioral sensitization (Lewis et al., 2020). Specifically, the activation of D2Rs on cholinergic interneurons inhibit cholinergic activity thus permitting sustained locomotor activity demonstrated in psychostimulant-induced sensitization (Lewis et al., 2020). Furthermore, spinophilin has been shown to be expressed in cortical parvalbumin-containing interneurons, but no data has been shown for cholinergic interneurons (E. Chris Muly et al., 2004). However, spinophilin has been shown to directly bind to D2Rs on the IC3 loop via its GPCR binding domain (Sarrouilhe et al., 2006; Smith et al., 1999). Furthermore, the phosphorylation status at a Thr site on the IC3 loop of D2Rs mediate surface expression via a receptor internalization mechanism (Cho et al., 2010). Together these data position spinophilin as a potential mediator of D2R signaling dynamics within the cholinergic interneuron. It is possible that loss of spinophilin in cholinergic interneurons may recapitulate the global KO data or more likely a combination of spinophilin deletion in iMSN and cholinergic interneurons.

3.5 Conclusion

A sensitizing regimen of amphetamine did not induce the appropriate behavioral response in the global spinophilin KO mice, such that they did not demonstrate locomotor

sensitization (**Figure 16**) (Morris et al., 2018). In addition, loss of spinophilin in either iMSNs or dMSNs did not recapitulate the deficits observed in the global spinophilin KOs and instead amphetamine-induced locomotor sensitization was intact (**Figure 17-20**). The hyperlocomotion induced by psychostimulants is mediated by increases in dopaminergic tone within the striatum (Asser & Taba, 2015; Barbera et al., 2016; Beaulieu et al., 2006; Durieux et al., 2012; Ikemoto, 2002; Natarajan & Yamamoto, 2011). In addition, the stimulation of both D1Rs and D2Rs produce robust locomotion (Asser & Taba, 2015; Korchounov, 2008; Waszczak et al., 2002). However, global loss of spinophilin still produced an initial hyperlocomotion on par with control mice, but the global spinophilin knockout mice did not sensitize to amphetamine (**Figure 16**) (Morris et al., 2018). These data suggest that loss spinophilin in other cell-types within the striatum may play a critical role in amphetamine-induced sensitization. Further research is needed to understand which cell type or cell types are regulating the whole-body spinophilin KO data.

Chapter IV: Molecular Mechanisms Underlying Psychostimulant-Mediated Striatal Spinophilin Function

4.1 Introduction

Psychostimulant drug abuse is becoming increasingly popular and costly globally (Ashok et al., 2017; Degenhardt & Hall, 2012). Psychostimulant drugs of abuse, such as methamphetamine, amphetamine, and cocaine, have been associated with dopamine (DA) receptor dysfunction, improper synaptic transmission, and other neuronal perturbations that may contribute to addiction pathology (Burke & Miczek, 2014; Centonze et al., 2002; Chiodi et al., 2014; Gerdeman et al., 2003; Sanchez-Ramos, 2015; Ungless et al., 2001). Psychostimulants drive hyper-dopaminergic signaling within the striatum by increasing DA concentrations and enhancing DA transmission (Cass et al., 1992; Dela Peña et al., 2015; Di Chiara & Imperato, 1988; Siviy et al., 2015; Volkow et al., 1999). When low doses of psychostimulants are administered chronically, response to the drug also increases, causing progressive potentiation of motor programs which eventually plateau, a process known as behavioral sensitization (**Figure 14**) (P. W. Kalivas & J. Stewart, 1991; Robinson & Becker, 1986; Steketee & Kalivas, 2011). Thus, DA plays a critical role in basal ganglia regulated motor programs (Bostan & Strick, 2018; Gremel & Lovinger, 2017; Groenewegen, 2003; Natarajan & Yamamoto, 2011).

The striatum is thought to play a significant role in mediating psychostimulant drug addiction/abuse (Gremel & Lovinger, 2017; Yager et al., 2015). The striatum is divided into two regions either the dorsal or ventral striatum (which includes the olfactory tubercle as an extension of the ventral striatum). Furthermore, the ventral

striatum is thought to mediate the rewarding features of psychostimulants (Ikemoto, 2002, 2003, 2007; Ikemoto et al., 2005). The dorsal striatum is subdivided into the dorsal medial striatum (DMS) and the dorsal lateral (DLS) striatum. The DMS is postulated to mediate associative tasks (DMS) thereby modulating goal-directed behavior, whereas the DLS regulates sensorimotor function mediating motor and habitual actions (Balleine & O'Doherty, 2010; Burton et al., 2015; Stalnaker et al., 2012). Together, the dorsal striatum is critical for decision making and is paramount in delineating how the striatum mediates/influences movement. The dorsal striatum is innervated by dopaminergic projections arising from the SN and has been functionally described as a modulator of motor domains specifically involving action selection and initiation (Balleine & O'Doherty, 2010; Graybiel, 2008; Graybiel & Grafton, 2015). However, studies also suggest that there is significant overlap in motor and reward functional domains within the striatum (Belin & Everitt, 2008; Kravitz & Kreitzer, 2012). Approximately 90–95% of the neuronal populations within the striatum are GABAergic MSNs. There are two MSN subtypes within the striatum that are characteristically distinct based on physiological and structural properties, as well as differential expression of DA receptor subtypes and neuropeptide hormones (Gerfen et al., 1990; Gerfen & Young, 1988; Lanciego et al., 2012). Differential expression of DA receptors allows for differential signaling within striatal MSNs and studies have shown that characteristics and behaviors associated with striatal specific pathological maladies can occur when there is an imbalance in the activity and/or signaling between the two MSN classes (Allichon et al., 2021; Bertran-Gonzalez et al., 2008; Lobo & Nestler, 2011). Activation of dMSNs increase PKA signaling (**Figure 6**) and act to enhance basal ganglia related motor

programs (Allen et al., 2006; Bateup et al., 2010; Mink, 1996; Price et al., 1999; Snyder et al., 2000; Xue et al., 2017). Conversely, iMSNs decrease PKA signaling (**Figure 6**) and are thought to inhibit inappropriate motor actions (Bateup et al., 2010; Durieux et al., 2009; Mink, 1996). Thus, modulation of dopaminergic signaling can drive the opposing functions of dMSNs and iMSNs (Bateup et al., 2010; Durieux et al., 2012; Surmeier et al., 2007; Surmeier et al., 2010).

Proper MSN signaling is facilitated by reversible protein phosphorylation is which contributes to competent neuronal signaling, communication, and synaptic plasticity. To achieve proper signaling, serine/threonine kinases phosphorylate substrates utilize specific consensus sites; however, serine/threonine phosphatases, such as PP1, associate with targeting proteins to attain specificity (Foley et al., 2021; Peti et al., 2013; Woolfrey & Dell'Acqua, 2015). The most abundant targeting protein for PP1 in the PSD is spinophilin (Kelker et al., 2007; MacMillan et al., 1999; E. Chris Muly et al., 2004). Spinophilin acts as a scaffolding protein by targeting PP1 to specific substrates (**Figure 8**); however, spinophilin can also inhibit the activity of PP1, driving changes in synaptic strength and plasticity (Allen et al., 1997; Foley et al., 2021; Morishita et al., 2001; Ragusa et al., 2010; Yan et al., 1999). Furthermore, spinophilin is enriched in the PSD of dendritic spines, and is essential for proper dendritic spine function by regulation of critical dendrite properties (Allen et al., 1997; Feng et al., 2000; E. Chris Muly et al., 2004; E. C. Muly et al., 2004). Changes in MSN dendritic spine density and morphology, perturbations in synaptic transmission, and concomitant aberrant dopaminergic signaling are all major contributors to striatal disease states such as psychostimulant drug addiction (Allichon et al., 2021; Areal et al., 2019; Surmeier et al., 2007; Surmeier et al., 2010;

Yager et al., 2015). In addition, psychostimulant administration, which drives hyper-dopaminergic responses was shown to increase spinophilin expression in the striatum (Boikess & Marshall, 2008; Boikess et al., 2010). Dopaminergic signaling regulates kinase activity (**Figure 6**), such as PKA and CaMKII. Spinophilin can be phosphorylated by PKA and CaMKII within its actin-binding domain (**Figure 8**) and phosphorylated spinophilin is known to decrease its binding to F-actin and potentially limit its coupling to the PSD (Grossman et al., 2004; Hsieh-Wilson et al., 2003). DA depletion, which decreases dopaminergic signaling in the striatum, decreases spinophilin interactions within the striatum. Specifically, there were increases in spinophilin binding to PP1; however, the interactions of spinophilin with a plurality of spinophilin-associated proteins (SpAPs) were decreased (Brown et al., 2008; Hiday et al., 2017). Previously, we showed that whole-body spinophilin knockout (KO) mice do not undergo d-amphetamine-induced behavioral sensitization (Morris et al., 2018). However, how excessive DA signaling, as occurs following psychostimulant sensitization, modulates spinophilin interactions is unclear. Here we show that in contrast to DA depletion, amphetamine sensitization increases a majority of striatal spinophilin interactions after a sensitizing regimen of amphetamine. Moreover, in our preliminary studies using a novel transgenic mouse line that allows for Cre-dependent expression of a hemagglutinin (HA)-tagged form of spinophilin, we observed both pan-MSN and putative cell type-specific alterations in spinophilin interactions following amphetamine treatment. In addition, global loss of spinophilin drives aberrant changes in the expression and phosphorylation state of critical post-synaptic proteins that facilitate proper MSN signaling. Together,

these data delineate alterations in direct and indirect spinophilin interactions that may contribute to psychostimulant-induced pathologies.

4.2 Materials and Methods

4.2.1 Animals

(*Proteomics*) Adult (P59-P87) A2A- or D1Cre HA-tagged spinophilin mice were used for mass spectrometry (**Table 1**). (*Immunoblotting Studies*) Adult (P85–P120) mice were used for immunoblotting analysis of HA spinophilin or Adult (P56-P64) old whole-body spinophilin knock-out ((B6N(Cg)-*Ppp1r9b*$^{tm1.1(KOMP)Vlcg}$/J) (KO), and wild-type (WT) C57BL/6 mice were used to investigate protein expression changes in sensitized animals. In addition, one adult P90 WT and one adult spinophilin KO mouse was used to validate a subset of spinophilin interactions for the proteomics data specifically.

4.2.2 Generation of cell-specific HA-tagged spinophilin mice

A human, HA-tagged spinophilin construct (Hiday et al., 2017) containing a P2A sequence and the mNeptune 3 protein were assembled into the pBigT vector between the ClaI and SacI restriction sites (**Figure 21A**). Gene files were assembled in SnapGene (GSL Biotech, Chicago, IL, USA) or Vector NTI (ThermoFisher Scientific, Waltham,

Eartag	Sex	Condition	Genotype	Cre	Initial Weight	Final Weight	Birth Date	Sacrifice Date
2450	M	Saline	Het/Cre+	D1	23.8	25.0	31 January 2018	30 March 2018
2452	M	Treated	Het/Cre+	D1	22.4	23	31 January 2018	30 March 2018
2453	M	Treated	tdHet/HA-Het/Cre+	D1	23.0	23.3	31 January 2018	30 March 2018
2454	M	Saline	Het/Cre+	D1	22.3	22.8	31 January 2018	30 March 2018
2390	F	Saline	Het/Cre+	A2A	22.9	22.8	3 January 2018	30 March 2018
2393	F	Treated	Het/Cre+	A2A	20.9	20.8	3 January 2018	30 March 2018
2443	F	Saline	Het/Cre+	D1	21.5	21.7	29 January 2018	30 March 2018
2444	F	Treated	Het/Cre+	D1	19.6	19.8	29 January 2018	30 March 2018

Table 1: **Proteomics Subjects**. Animals used for proteomics studies. Sex, genotype, weight of animals used for proteomics studies. Adopted/Modified from (Watkins et al., 2018). Created with BioRender.com.

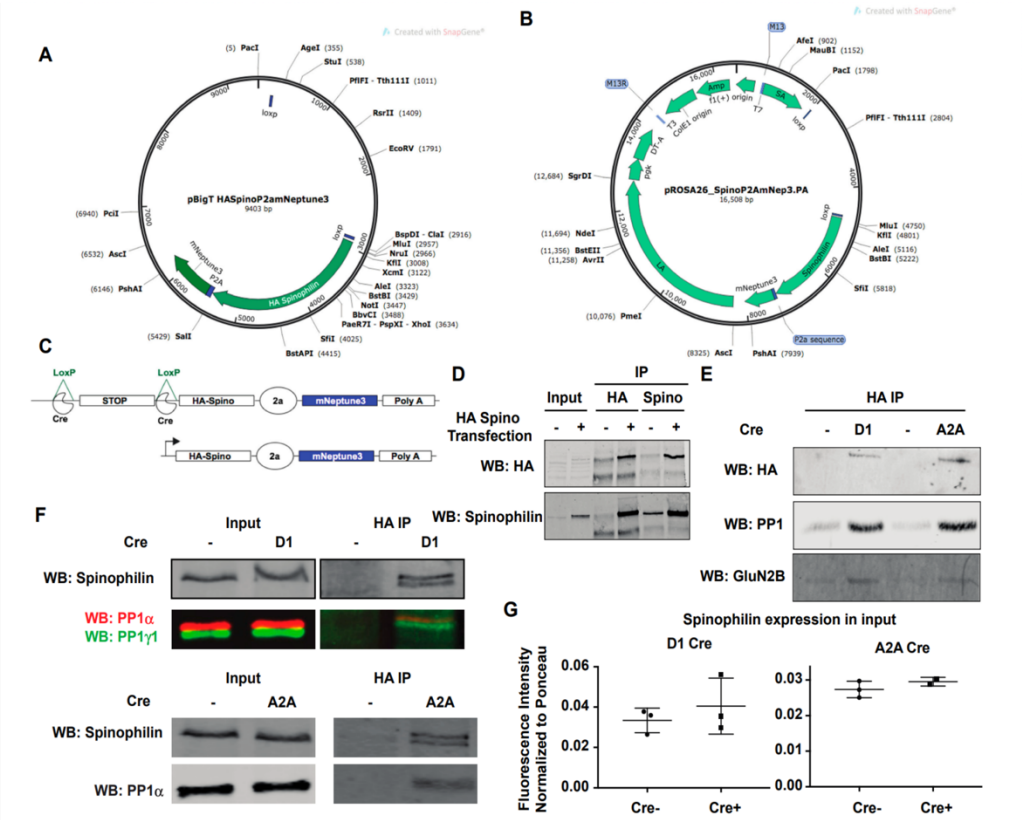

Figure 21: Generation and Characterization of Cre-expressing HA-tagged Human Spinophilin Mice. (A) A construct containing DNA encoding HA-tagged human spinophilin with a P2A sequence and mNeptune3 fluorescent protein was cloned into the pBIGT vector that contains a floxed-stop sequence. (B) The construct encoding the floxed-stop sequence and the HA-spinophilin-P2A-mNeptune 3 sequence was subcloned into the ROSA targeting vector pROSA_26.PA. (C) The modified ROSA vector was used for generation of the targeted transgenic mice. (D) Striatal cells were transfected without or with HA-tagged human spinophilin. Lysates were immunoprecipitated with either an HA or spinophilin antibody and immunoblotted with an HA antibody or a spinophilin antibody. HA-spinophilin was selectively detected when it was overexpressed. € Mice express HA-tagged spinophilin upon crossing with Cre recombinase expressed in the direct pathway (D1) or indirect pathway (A2A) medium spiny neurons. (F) Spinophilin

and protein phosphatase 1 immunoblots of inputs and HA-immunoprecipitates from HA spinophilin mice crossed with D1 or A2A Cre-recombinase-expressing mice. (G) Mice expressing HA-spinophilin had non-significant increases in total spinophilin expression. Modified/Adopted Figure from (Watkins et al., 2018) Created with BioRender.com.

MA, USA). The insert was then subcloned into the AscI/PacI sites on the pROSA26.PA vector (**Figure 21B**) for generation of targeted embryonic stem cells (ES) cells. pROSA26.PA vector was linearized with AscI and injected into SV129 ES cells by the Vanderbilt Transgenic Mouse/ESC Shared Resource. These ES cells were transferred into pseudo-pregnant C57Bl6/J females and chimeric pups were born from two of these clones (2D4 and 2E12). Chimeras were transferred from the Vanderbilt Transgenic Mouse/ESC Shared Resource to the mouse colony at IUPUI. One clone was maintained in house. Mice were backcrossed at least six generations onto a C57Bl6/J background. Mice were subsequently crossed with either the Drd1a-Cre line or onto an A2A-Cre line (Gerfen et al., 2013; Gong et al., 2007) that were on the C57Bl6/J background. For proteomics, mice expressing a single copy of spinophilin knocked-in to the ROSA locus were used. For immunoblotting, mice expressing HA-spinophilin knocked into one or both copies of the ROSA locus were used. For those expressing a single copy of spinophilin, the other ROSA allele was either WT or had a flox-stop tdTomato reporter sequence inserted (Jackson laboratories Stock #007914, Bar Harbor, ME, USA). All animal studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals as disseminated by the U.S. National Institutes of Health and were approved by Indiana University-Purdue University School of Science Animal Care and Use Committees (Approval #SC270R).

4.2.3 Brain Tissue Lysis

Whole mouse striatum (including both dorsal and ventral (accumbens) striatum or olfactory tubercle) was dissected from mice after live decapitation. Striatal tissue was

homogenized and sonicated in either 1mL (*Proteomics*) or 1.25mL (*Immunoblotting*) of ice cold low ionic strength lysis buffer containing 2 mM Tris-HCl, 1 mM DTT, 2 mM EDTA, 1% Triton X-100, 1X protease inhibitor cocktail (Bimake, Houston, TX, USA), and phosphatase inhibitors (20 mM sodium fluoride, 20 mM sodium orthovanadate, 20 mM *β*-glycerophosphate, and 10 mM sodium pyrophosphate; Sigma-Aldrich or ThermoFisher Scientific (Waltham, MA, USA)). Homogenates were incubated for 15 min at 4 °C and then centrifuged at 13,600× *g* for 10 min. The collected supernatant was mixed with Laemmli sample buffer to generate the inputs or subjected to immunoprecipitation.

4.2.4 Transfections

Mouse STHdhQ7/7 striatal cell line (a kind gift from Dr. Gunnar Kwakye, Oberlin College, Oberlin, OH, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM) that contained 10% fetal bovine serum, 1% GlutaMAX™ (ThermoFisher Scientific), 400 µg/mL G418-Sulfate (Geneticin) (ThermoFisher Scientific), 100 U/mL penicillin, and 100 µg/mL streptomycin. Culture plates were incubated at a constant 33 °C and 5% CO_2 in myTemp Mini CO_2 digital incubator (Benchmark Scientific; Edison, NJ, USA). Cells were transfected overnight with 2 µg of HA-tagged human spinophilin and PolyJet reagent (SignaGen Laboratories, Gaithersburg, MD, USA) per the manufacturers' instructions. Cells were lysed in the low-ionic strength Tris buffer.

4.2.5 Immunoprecipitations

Striatal lysates were immunoprecipitated with an HA-epitope antibody or spinophilin antibody. 3 µg of goat HA polyclonal antibody (Bethyl Laboratories, Montgomery, TX, USA, A190-238A) or 5 µg goat spinophilin polyclonal antibody (Santa Cruz Biotechnology, Dallas, TX, USA, SC14774) were incubated at 4 °C with 750–800 µL (75–80%) of total striatal lysate overnight. Striatal cell lysates were immunoprecipitated with 1.6 µg of a sheep spinophilin antibody (ThermoFisher Scientific). The following day, protein G magnetic beads (DynaBeads, ThermoFisher Scientific) were added, and the mixture was incubated for 2 h. Beads were washed three times by magnetic separation in an immunoprecipitation wash buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.5, 0.5% (*v*/*v*) Triton X-100). For immunoblotting, beads were resuspended in 2X sample buffer. For Tandem Mass Tag (TMT) labeling, beads were subsequently washed three times in PBS by centrifugation. Washed beads were submitted for tryptic digestion and each sample was labeled with an isobaric tandem mass tag to allow for quantitation.

4.2.6 Immunoblotting

Immunoprecipitates and inputs were separated by SDS-PAGE and blotted with specific primary or secondary antibodies (**Table 2**). Immunoblots were imaged using either Odyssey CLX or Odyssey M and fluorescence intensity measurements were made using Image Studio or Imperia respectively (LI-COR Biosciences, Lincoln, NE). Immunoblotting was performed as previously described (Edler et al., 2018; Morris et al., 2018; Watkins et al., 2018).

Antibody	Dilution	Source	Identifier
Primary Antibodies			
CAMKIIa (6G9) Ms	1:1000	Santa Cruz Biotechnology	sc-32288
D2 (B-10) Ms	1:1500	Santa Cruz Biotechnology	SC-5303
DARP 32 (19A3) Rb	1:1000	Cell Signaling Technology	2306S
GluA1 (D4N9V) Rb	1:1000	Cell Signaling Technology	13185S
GluA2 (E1L8U) Rb	1:1000	Cell Signaling Technology	13607S
GluN1 (D65B7) Rb	1:1000	Cell Signaling Technology	5704S
GluN2A Rb	1:1000	Cell Signaling Technology	4205S
GluN2B (D8E10) Rb	1:1000	Cell Signaling Technology	14544S
HA Gt (discontinued) (IP)	*see methods	Bethyl Laboratories	A190-238A
HA Rb	1:500	Bethyl Laboratories	A190-208A
mGluR5 Rb	1:1000	Millipore Sigma	AB5675
mGluR5, clone N75/33 Ms	1:1500	Millipore Sigma	MABN540
Neurabin I Rb (discontinued)	1:1000	Sigma-Aldrich*	N 4412
Phospho-CaMKII (22B1) Ms	1:1000	Santa Cruz Biotechnology	sc-32289
Phospho-DARPP-32 (Thr34) (D27A4) Rb	1:1000	Cell Signaling Technology	12438S
Phospho-GluA1 (A5O2P) Ser831 Rb	1:1000	Cell Signaling Technology	75574S
Phospho-GluA1 (D10G5) Ser845 Rb	1:1000	Cell Signaling Technology	8084S
Phospho-GluN2B S1284	1:1000	Cell Signaling Technology	5355S
PP1alpha (E-9) Ms	1:1000	Santa Cruz Biotechnology	sc-7482
PP1gamma (C-19) Gt	1:1000	Santa Cruz Biotechnology	sc-6108
PSD-95 (D27E11) XP Rb	1:1000	Cell Signaling Technology	3450S
SAP102 (A7R8L) Rb	1:1000	Cell Signaling Technology	47421S

Antibody	Dilution	Source	Identifier
Spinophilin (A-20) Gt (discontinued) (IP)	*see methods	Santa Cruz Biotechnology	sc-14774
Spinophilin (E1E7R) Rb	1:500	Cell Signaling Technology	14136S
Spinophilin Sheep	*see methods	ThermoFisher Scientific	PA5-48102
Secondary Antibodies			
Donkey anti-rabbit (H+L) Alexa Fluor 790	1:10,000	Jackson Immunoresearch	711-655-152
Donkey anti-mouse (H+L) Alexa Fluor 790	1:10,000	Jackson Immunoresearch	715-655-151
Donkey anti-sheep (H+L) Alexa Fluor 790	1:10,000	ThermoFisher Scientific	A11374
Donkey anti-goat (H+L) Alexa Fluor 680	1:10,000	ThermoFisher Scientific	A21084
Donkey anti-mouse (H+L) Alexa Fluor 680	1:10,000	ThermoFisher Scientific	A10038

Table 2: **Antibody List**. Antibodies (primary and secondary), dilution, purchased company and catalog identifier.

4.2.7 d-Amphetamine Sensitization

Mice received daily intraperitoneal (i.p) injections of d-amphetamine at 3.0 mg/kg (Sigma-Aldrich, St. Louis, MO, USA) or saline (10 mL/kg) for five consecutive days and returned to their home cage. Mice were then sacrificed and striata dissected 48-72 h after the last injection.

4.2.8 Proteomics

Following washes, immunoprecipitated samples on beads were reduced with 5 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and alkylated with 10 mM chloroacetamide (CAM). Beads were then incubated with Trypsin Gold (Promega) at 37 °C overnight. Digested samples were cleaned up using a Waters Sep-Pak C18 plate per manufacturer's instructions.

For TMT labeling, tryptic peptides from each individual condition were labeled with eight different isobaric TMT tags using 8 of a 10-plex TMT kit (ThermoFisher Scientific) and following manufacturer's instructions. Following individual labeling, samples were mixed and separated by HPLC and subjected to mass spectrometry.

For HPLC and mass spectrometry analysis, digested peptides were loaded onto an Acclaim PepMap C18 trapping column and eluted on a PepMap C18 analytical column with a linear gradient from 3% to 35% acetonitrile (in water with 0.1% formic acid) over 120 min in-line with an Orbitrap Fusion Lumos Mass Spectrometer (ThermoFisher Scientific). Raw files generated from the run were analyzed using Thermo Proteome Discoverer (PD) 2.2. SEQUEST HT (as a node in PD 2.2) was utilized to perform

database searches as previously described (Smith-Kinnaman et al., 2014) with a few modifications: Trypsin digestion, two maximum missed cleavages, precursor mass tolerance of 10 ppm, fragment mass tolerance of 0.8 Da, fixed modifications of +57.021 Da on cysteine, +229.163 on lysines and peptide N-termini, and a variable modification of +15.995 Da on methionine. The spectral false discovery rate (FDR) was set to ≤1% as previously described (Mosley et al., 2011). The FASTA database used was a mouse proteome downloaded from Uniprot on January 9, 2017, with the addition of 72 common contaminants.

4.2.9 Pathway Analysis

Proteins that were increased in HA-spinophilin immunoprecipitates isolated from amphetamine treated animals were analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) Bioinformatics Resource (version 6.8; https://david.ncifcrf.gov/, National Cancer Instutitue at Frederick, MD, USA 69 70). Proteins were analyzed using the Kyoto encyclopedia of genes and genomes (KEGG) and gene ontology (GO) pathway databases. In addition, (Search Tool for the Retrieval of Interacting Genes/Proteins) STRING database (http://www.string-db.org) was used to display KEGG pathway protein networks.

4.2.10 Statistics

To compare saline to amphetamine treatment across all groups combined, a t-test was performed to compare spinophilin abundance in the amphetamine vs. saline treated

samples. A non-adjusted t-test and a t-test adjusted for multiple comparisons (using the Holm-Sidak method) were performed to compare the abundance of the proteins isolated from amphetamine compared to saline-treated samples both non-normalized and normalized. For normalization for PCA analysis and protein abundance, we divide the abundance of the individual protein in the individual sample by the total peptide or total spinophilin abundance detected in that sample. Given that there was an N of 1 in some of the sub-categories (e.g., A2A male and D1 female) the study was not powered nor intended to make statistical conclusions and these results are a qualitative display of sex- and cell-specific protein interactions. For immunoblotting, Two-way ANOVA with repeated measures was performed to determine effect of loss of spinophilin on amphetamine treated subjects compared with appropriate controls. A post-hoc Tukey or Šídák's multiple comparison test was performed when genotype, treatment, or interaction had a p-value > 0.05. If $p > 0.05$, multiple comparisons were only performed within columns and/or rows that had a significant ANOVA effect.

4.3 Results

Generation of HA-Tagged Spinophilin Mice

We created constructs that encoded HA-tagged human spinophilin (Hiday et al., 2017) along with a P2A sequence and a far-red fluorescent protein (mNeptune3 (Chu et al., 2014)). Mice were generated from these constructs by the Vanderbilt Transgenic Mouse/ESC Shared Resource (see methods). When crossed with Cre-expressing mice, these animals express HA-tagged human spinophilin under control of the ROSA promoter (**Figure 21C**). When crossed with mice expressing Cre recombinase under

control of the *Drd1a* gene or the *Adora2a* gene, we were able to detect HA signal in HA immunoprecipitates by immunoblotting (**Figure 21D,E**). Furthermore, we were able to detect known spinophilin interacting proteins PP1 and GluN2B (Allen et al., 1997; Baucum et al., 2013; Baucum et al., 2010; Colbran et al., 1997) in the HA immunoprecipitates isolated from Cre-expressing lines (**Figure 21E**). Less PP1 and GluN2B co-precipitated from the Cre-negative animals (**Figure 21E**). Moreover, a spinophilin antibody also detected a band in the HA immunoprecipitates isolated from Cre-expressing, but not Cre-negative mice (**Figure 21D,F**). Of note, we detected a doublet in the HA-immunoprecipitates when immunoblotting for spinophilin isolated from the HA-spinophilin expressing mice, as well as a striatal cell line transfected with an HA-spinophilin construct (**Figure 21F**). This is not surprising as spinophilin is thought to homo-dimerize and this suggests that the human HA-spinophilin is complexing with the endogenous, mouse spinophilin. However, there was no significant difference in the amount of total spinophilin in the mice expressing HA-spinophilin, suggesting a low overexpression of spinophilin (**Figure 21G**). Moreover, given the low expression of epitope tagged spinophilin and fluorescent protein, we were unable to detect either HA-tagged protein or fluorescent protein by immunohistochemistry (data not shown).

Amphetamine Modulates Spinophilin Expression and Interactions

DA signaling within the striatum modulates MSN activity and signaling (Gerfen & Surmeier, 2011; Hu & Wang, 1988). Amphetamine increases the release of DA at dopaminergic terminals synapsing on MSNs (Dela Peña et al., 2015; Di Chiara &

Imperato, 1988; Siviy et al., 2015). Our previous studies show that DA depletion alters the spinophilin interactome (Hiday et al., 2017) and that spinophilin KO mice do not undergo amphetamine-induced locomotor sensitization (Morris et al., 2018). However, how spinophilin normally contributes to synaptic changes associated with amphetamine-dependent striatal changes is unclear. As spinophilin targets PP1 to regulate synaptic protein phosphorylation, in order to identify potential spinophilin-dependent synaptic protein targets that are regulated by spinophilin following amphetamine sensitization, we utilized our HA-tagged spinophilin mice (**Figure 21**) to measure spinophilin interactions in saline- or amphetamine-treated mice expressing spinophilin in D1 DA or A2A adenosine-receptor containing neurons of the striatum. Mice were injected with 3 mg/kg amphetamine every day for five days and sacrificed 72 h after the final amphetamine treatment. Striatal lysates were immunoprecipitated with an HA antibody, digested with trypsin, labeled with TMTs, and analyzed by mass spectrometry (**Figure 22A**). A total TMT abundance for spinophilin was detected in all conditions. As human and mouse spinophilin differ by fewer than 30 amino acids and as spinophilin homo-dimerizes we searched only the mouse database. Forty-eight spectral counts matching spinophilin were detected across all eight samples. Based on the unnormalized abundance of the TMT tag from the different samples, we observed more spinophilin in the amphetamine-treated compared to control treated samples (**Figure 22B**). A principal component analysis from all proteins (1454) of the individual samples, normalized to total peptide amount, revealed that 46.2% of the total variability is due to amphetamine treatment (**Figure 22C).** We next evaluated changes in the TMT tag abundance ratios (amphetamine/saline)

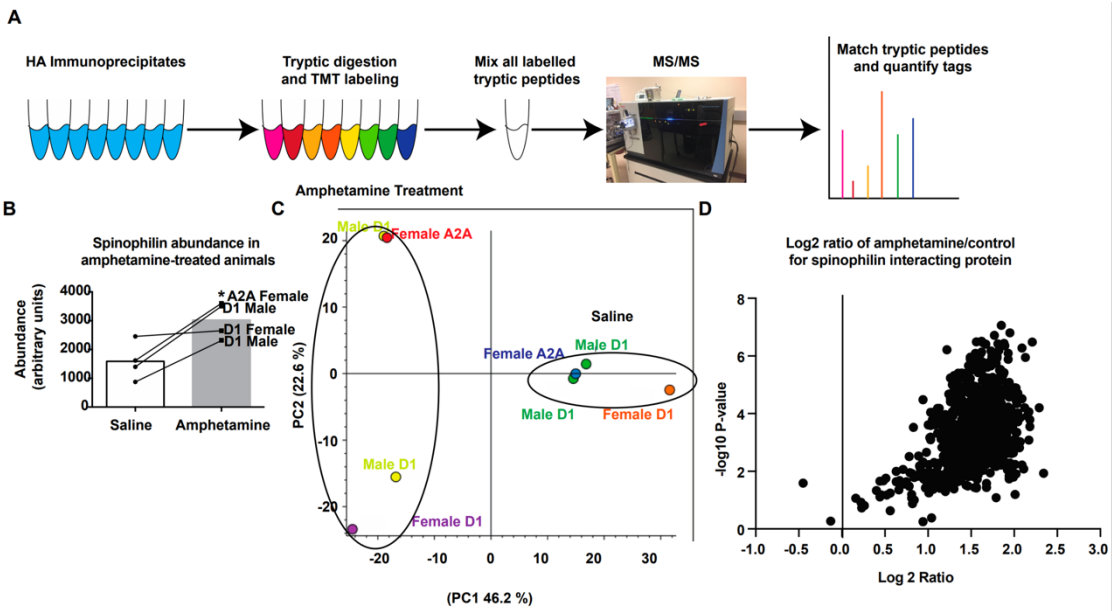

Figure 22: **Quantitation of Spinophilin Complexes isolated from MSNs using TMT Analysis**. (A) Striatal lysates isolated from male or female mice expressing HA spinophilin under the control of D1 or A2A promoters and treated with saline or amphetamine were immunoprecipitated with an HA antibody, digested with trypsin, labelled with eight different TMT tags, mixed and analyzed by mass spectrometry (MS/MS). (B) A higher intensity of TMT reporter abundance matching spinophilin was observed in amphetamine-treated compared to saline treated animals (*t*-test; * $p < 0.05$). (C) Principal component analysis of individual samples normalized to total peptide amount within each sample. (D) A volcano plot showing a majority of the protein have increased abundance in HA immunoprecipitates isolated from amphetamine treated animals. Adopted/Modified from (Watkins et al., 2018). Created with BioRender.com.

of the spinophilin interacting proteins. For this, we eliminated all contaminant proteins and only included those proteins detected in all eight samples (e.g., with all eight tags).This led to the detection of 984 total proteins in the HA-spinophilin immunoprecipitates. We plotted these unnormalized values using a volcano plot with Log2 abundance ratio on the X-axis and −log10 *p*-value (*t*-test, non-adjusted) on the Y-axis (**Figure 22D**). All but two proteins showed an increased abundance in spinophilin immunoprecipitates isolated from amphetamine compared to saline treated samples.

Regulation of Spinophilin Interactions by Amphetamine

Given that spinophilin abundance was increased in immunoprecipitates isolated from amphetamine treatment compared to saline-treated samples, to determine if the increased association of spinophilin with interacting proteins was due exclusively to increased spinophilin levels, we normalized the abundance of each individual interacting protein to the abundance of spinophilin in the corresponding sample. Moreover, we only used those proteins that contained at least eight peptide spectral matches (PSMs) as these would average 2 PSMs per condition. We detected 423 total proteins across all conditions that met these criteria. Of these proteins, 134 were unchanged (Log2 ratio −0.5 to +0.5), three had a decreased association (<Log2 Ratio −0.5), and 286 had an increased association (>Log2 Ratio +0.5) with spinophilin. Those proteins with a decreased interaction ratio of <−0.5 and increased interaction ratio of >1.0 are shown in (**Table 3**). We performed a second PCA analysis of the data normalized to spinophilin for those peptides having eight PSMs or more (**Figure 23A**). This mode of analysis decreased the

Description	# PSMs	Normalized Abundance Ratio (Treatment)/(Control)	Normalized Abundance Ratio (log2): (Treatment)/(Control)
Decreased Interactions			
E3 ubiquitin-protein ligase XIAP	22	0.69	−0.54
Disks large homolog 3	143	0.67	−0.58
Granulins	10	0.36	−1.47
Increased Interactions			
Myelin proteolipid protein	38	2.28	1.19
Hemoglobin subunit alpha	17	2.24	1.16
ADP/ATP translocase 2	36	2.23	1.16
Clathrin light chain A	17	2.13	1.09
Adenylyl cyclase-associated protein 2	8	2.10	1.07
MCG10343, isoform CRA_b	35	2.08	1.05
Tubulin alpha-1B chain	54	2.07	1.05
Tubulin alpha chain (Fragment)	54	2.03	1.02
Cytochrome c oxidase subunit NDUFA4	13	2.03	1.02
Profilin-2	14	2.02	1.02
Reticulon (Fragment)	20	2.02	1.01
Myelin-oligodendrocyte glycoprotein	18	2.01	1.01
Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	28	2.00	1.00

Table 3: Spinophilin interacting proteins that had altered abundance following amphetamine treatment. HA spinophilin was immunoprecipitated from saline and amphetamine-treated D1 Cre and A2A Cre mice. The abundance of the individual proteins was normalized to the abundance of spinophilin. A ratio of the abundance of proteins isolated from the amphetamine treated over the saline treated mice was generated. A subset of spinophilin interacting proteins that had at least eight spectral counts (PSMs) and had a decreased (<0.5) or increased (≥1.00) log2 ratio is shown. Adopted/Modified from (Watkins et al., 2018). Created with BioRender.com.

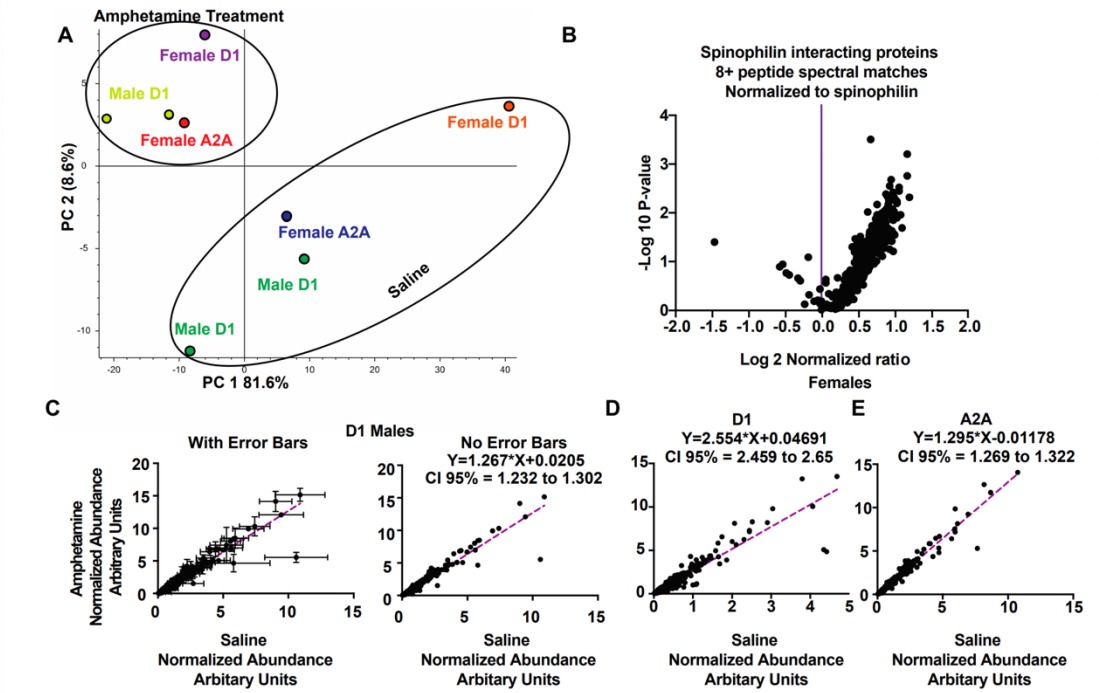

Figure 23: Greater Abundance of Spinophilin Interacting Proteins in Amphetamine-treated Animals Occurs Across Sexes and Cell-types. (A) Principal component analysis of individual samples normalized to spinophilin abundance within each sample and filtered for eight or more PSMs. (B) A volcano plot showing a majority of the proteins have increased abundance in HA immunoprecipitates isolated from amphetamine treated animals when normalized to the amphetamine-dependent increase in spinophilin abundance. (C) A plot of the abundance of spinophilin interacting proteins isolated from male, D1 Cre expressing animals and normalized for spinophilin expression and quantified from treated (Y-axis) or control (X-axis) samples. Left panel shows mean ± standard deviation, the right panel just shows the mean of the two values. (D) A plot of the abundance of spinophilin interacting proteins isolated from female, D1 Cre expressing animals and normalized for spinophilin expression and quantified from treated (Y-axis) or control (X-axis) samples. € A plot of the abundance of spinophilin interacting proteins isolated from female, A2A-Cre expressing animals and normalized for

spinophilin expression and quantified from treated (Y-axis) or control (X-axis) samples. Adopted/Modified from (Watkins et al., 2018). Created with BioRender.com.

variability within the amphetamine treatment group but increased the variability in the saline treatment group. We plotted these normalized values using a volcano plot with log2 abundance ratio on the X-axis and −log10 P-value (*t*-test, non-adjusted) on the Y-axis (**Figure 23B**). Therefore, even when normalized to spinophilin there is a higher number of proteins with an enhanced association with spinophilin compared to a decreased or no change in association.

We next evaluated the spinophilin interacting proteins from the different cell types and sexes. It is important to note that evaluation of these sub-categories (sex and genotype) are qualitative and no statistical inference can be made; however, these preliminary studies denote the importance of evaluating different sexes and cell types. We plotted the abundance of the amphetamine treated samples on the Y-axis and the abundance of the saline-treated samples isolated from D1 males (**Figure 23C**), D1 females (**Figure 23D**) and A2A females (**Figure 23E**). All data were normalized to spinophilin abundance. All three sets had a slope greater than 1, suggesting that there was greater abundance in the amphetamine treatment compared to the saline treatment. Of note, the D1 females had the greatest slope (M = 2.554), suggesting the greatest increased association in this group compared to the D1 males (M = 1.267) or A2A females (M = 1.269). Together, our data suggest that amphetamine treatment enhances synaptic protein interactions with spinophilin across multiple sexes and cell types.

To begin to delineate amphetamine-dependent regulation of specific spinophilin interactors in the different Cre lines, we generated a ratio of the abundance ratios from the D1 Cre animals to the A2A Cre animals. As stated above, it is important to note that, given the N of 1 in the A2A animals, these ratios are qualitative and no statistical

inference can be obtained; however, these data will inform novel lines of inquiry in future cell-specific studies. Those proteins with this ratio of ratios greater than 2 are shown in (**Table 4**). No interactions with a ratio of less than 0.5 (2-fold decrease) were detected.

Pathway and GO Analysis of Spinophilin Interacting Proteins Enhanced by Amphetamine

Using the DAVID Bioinformatics resource (Huang da et al., 2009; Huang et al., 2009), we performed the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis on the 286 proteins that had an increased association with spinophilin across all samples. 283 total proteins were detected from the list. A total of 94 pathways were detected including pathways associated with striatal function, including amphetamine addiction. The top 10 pathways also include other disease states associated with striatal dysfunction, such as Parkinson disease and Huntington disease (**Table 5**). We also used accession Uniprot ID for identified proteins from HA immunoprecipitates and inputted the data in bioinformatics STRING database (http://www.string-db.org) and analyzed using KEGG enrichment pathway mapping to reveal molecular interactions that overlap (**Figure 24**). We next evaluated these increased interactions in DAVID using gene ontology terms (GO). We evaluated Biological Processes (BP), Cellular Components (CC), and Molecular Function (MF). We detected a total of 247 BPs, 154 CCs, and 127 different MFs in the increased spinophilin interactors. Of these, 33, 55, and 31, respectively, were significantly enriched. For BPs, we observed a large number of metabolic processes, including ATP and NADH metabolism. We also observed vesicle trafficking processes, including synaptic vesicle endocytosis, vesicle-mediated transport, and endocytosis. For CCs, we observed known areas where spinophilin is enriched,

Description	PSMs	Female A2A Ratios	Female D1 Ratios	Male D1 Avg Ratios	Avg D1/A2A Ratios
Endophilin-A2	12	0.50	1.38	1.28	2.63
Cofilin-1	14	1.13	4.14	1.57	2.52
Malate dehydrogenase, mitochondrial	27	0.92	3.22	1.38	2.51
Calreticulin	8	0.87	3.15	1.11	2.46
Alpha-synuclein	21	1.06	3.56	1.61	2.43
Malate dehydrogenase, cytoplasmic	41	1.05	3.59	1.38	2.36
Glutamate dehydrogenase 1, mitochondrial	23	1.05	3.58	1.39	2.36
Fructose-bisphosphate aldolase	87	1.14	3.93	1.41	2.35
Protein disulfide-isomerase A3	9	0.93	3.19	1.13	2.31
Citrate synthase, mitochondrial	32	1.37	4.58	1.55	2.24
Synapsin-1	42	1.11	3.51	1.45	2.23
Tubulin polymerization-promoting protein	9	1.08	3.24	1.56	2.23
Nucleoside diphosphate kinase	12	1.20	3.92	1.39	2.21
Fascin	26	0.89	2.78	1.13	2.20
Cytochrome b-c1 complex subunit 1, mitochondrial	19	0.99	3.09	1.26	2.19
Carbonic anhydrase 2	14	1.22	3.75	1.54	2.18
Rab GDP dissociation inhibitor alpha	22	1.12	3.58	1.26	2.16
Protein kinase C and casein kinase substrate in neurons protein 1	18	1.10	3.36	1.30	2.12
Endophilin-A1	23	1.11	3.37	1.33	2.12
Cytochrome c oxidase subunit 5B, mitochondrial	13	1.17	3.52	1.43	2.12

Table 4: Spinophilin interacting proteins that had altered abundance ratios in D1-Cre animals compared to A2A-Cre Animals. HA spinophilin was immunoprecipitated from saline and amphetamine-treated D1-Cre and A2A-Cre mice. The abundance of the individual proteins was normalized to the abundance of spinophilin. A ratio of the abundance of proteins isolated from amphetamine-treated divided by saline-treated mice was generated. A second ratio comparing the amphetamine/saline ratios identified in the 3 D1 samples and the 1 A2A sample was generated. Those D1/A2A ratios ≥ 2.00 are shown. Adopted/Modified from (Watkins et al., 2018). Created with BioRender.com.

Term	Count	%	p Value	Bonferroni
mmu01200:Carbon metabolism	27	9.54	3.52×10^{-18}	7.04×10^{-16}
mmu05012:Parkinson's disease	29	10.25	2.34×10^{-17}	4.69×10^{-15}
mmu05016:Huntington's disease	32	11.31	9.23×10^{-17}	2.22×10^{-14}
mmu00190:Oxidative phosphorylation	26	9.19	4.42×10^{-15}	8.88×10^{-13}
mmu04721:Synaptic vesicle cycle	19	6.71	5.04×10^{-15}	9.99×10^{-13}
mmu00020:Citrate cycle (TCA cycle)	15	5.30	7.94×10^{-15}	1.60×10^{-12}
mmu05010:Alzheimer's disease	27	9.54	1.82×10^{-13}	3.65×10^{-11}
mmu01130:Biosynthesis of antibiotics	29	10.25	3.85×10^{-13}	7.70×10^{-11}
mmu04961:Endocrine and other factor-regulated calcium reabsorption	15	5.30	1.92×10^{-11}	3.84×10^{-9}
mmu00010:Glycolysis/Gluconeogenesis	16	5.65	5.11×10^{-11}	1.02×10^{-8}

Table 5: Top 10 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with proteins that have amphetamine-dependent increases in spinophilin. The 286 proteins that had an increased association with spinophilin (log2 ratio ≥ 0.5) were input into the DAVID Bioinformatics resource and analyzed using KEGG pathway analysis. The top 10 enriched pathways are shown. Adopted/Modified from (Watkins et al., 2018). Created with BioRender.com.

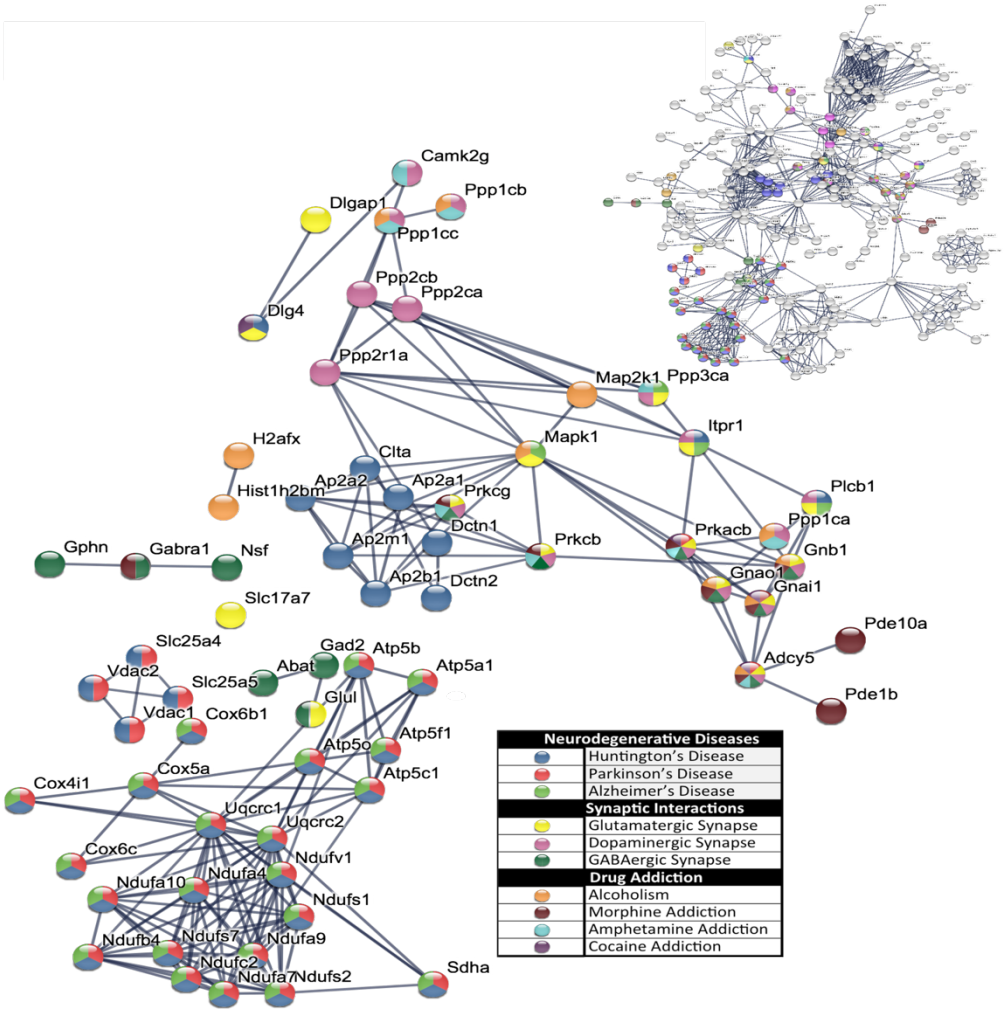

Figure 24: Amphetamine-dependent Striatal Spinophilin Interactome. The accession Uniprot ID for identified proteins from HA immunoprecipitates were inputted in bioinformatics STRING database (http://www.string-db.org) and analyzed using KEGG enrichment pathway. Selected proteins were ≥ 0.5 in abundance ratios (Treatment/Control) using (log_2) scale after being normalized to total spinophilin. Created with BioRender.com.

including membrane, postsynaptic density, dendrite, dendritic spine, and cytoskeleton. In addition, we matched other localizations where spinophilin has been implicated, such as synaptic vesicle membrane (Muhammad et al., 2015). For MFs, we observed known roles for spinophilin as a scaffold binding, protein complex binding, protein kinase binding, and actin filament binding. In addition, we observed novel putative roles for spinophilin, including GTPase binding, ATP binding, and syntaxin-1 binding. The top 10 pathways for BP, CC, and MF are shown in (**Table 6**).

Interactome Analysis of Spinophilin Interacting Proteins Enhanced by Amphetamine

We next wanted to organize spinophilin interacting proteins that were enhanced by amphetamine based on interactions and functional classifications. To do this, we utilized the string-db program (Szklarczyk et al., 2015; Szklarczyk et al., 2017). This allows for the pictorial representation of proteins and their interactions. To reduce the complexity of the submitted proteins, we used a high stringency confidence score (0.900) and removed any proteins that were not connected. We next grouped proteins into 12 categories based on known function and these interactions (**Figure 25**). These categories are: Metabolism, ATPases, vesicle trafficking, synaptic signaling, cytoskeleton, ribosomal and nuclear, scaffolding, heatshock, G-proteins and GTPases, semaphorin signaling, BBSome, and other.

Term	Count	%	PValue	Bonferroni
Biological Process				
GO:0006099~tricarboxylic acid cycle	13	4.59	3.85×10^{-15}	6.78×10^{-12}
GO:0006810~transport	68	24.03	1.55×10^{-12}	2.70×10^{-9}
GO:0006096~glycolytic process	11	3.89	8.12×10^{-11}	1.42×10^{-7}
GO:0006734~NADH metabolic process	8	2.83	1.11×10^{-10}	1.93×10^{-7}
GO:0046034~ATP metabolic process	11	3.89	2.57×10^{-10}	4.48×10^{-7}
GO:0015992~proton transport	12	4.24	7.90×10^{-10}	1.38×10^{-6}
GO:0015991~ATP hydrolysis coupled proton transport	10	3.53	8.81×10^{-10}	1.54×10^{-6}
GO:0050821~protein stabilization	15	5.30	7.11×10^{-9}	1.24×10^{-5}
GO:0015986~ATP synthesis coupled proton transport	8	2.83	2.98×10^{-8}	5.20×10^{-5}
GO:1904871~positive regulation of protein localization to Cajal body	6	2.12	3.79×10^{-8}	6.61×10^{-5}
Cellular Compartment				
GO:0043209~myelin sheath	92	32.51	3.70×10^{-120}	1.53×10^{-117}
GO:0070062~extracellular exosome	159	56.18	2.16×10^{-65}	8.91×10^{-63}
GO:0005739~mitochondrion	88	31.10	3.66×10^{-27}	1.51×10^{-24}
GO:0005829~cytosol	88	31.10	4.60×10^{-26}	1.90×10^{-23}
GO:0005737~cytoplasm	173	61.13	3.24×10^{-22}	1.34×10^{-19}
GO:0016020~membrane	178	62.90	6.47×10^{-22}	2.66×10^{-19}
GO:0014069~postsynaptic density	32	11.31	5.02×10^{-21}	2.07×10^{-18}
GO:0043005~neuron projection	40	14.13	6.70×10^{-21}	2.76×10^{-18}
GO:0043234~protein complex	46	16.25	1.63×10^{-19}	6.71×10^{-17}
GO:0005743~mitochondrial inner membrane	37	13.07	2.23×10^{-19}	9.20×10^{-17}
Molecular Function				
GO:0005515~protein binding	138	48.8	1.12×10^{-22}	6.18×10^{-20}
GO:0032403~protein complex binding	36	12.7	2.54×10^{-18}	1.40×10^{-15}
GO:0019901~protein kinase binding	36	12.7	1.24×10^{-15}	6.74×10^{-13}
GO:0044822~poly(A) RNA binding	55	19.4	4.52×10^{-14}	2.49×10^{-11}
GO:0000166~nucleotide binding	74	26.1	2.72×10^{-13}	1.50×10^{-10}
GO:0019904~protein domain specific binding	27	9.5	5.67×10^{-13}	3.13×10^{-10}
GO:0098641~cadherin binding involved in cell-cell adhesion	26	9.2	1.77×10^{-12}	9.79×10^{-10}
GO:0008022~protein C-terminus binding	22	7.8	1.30×10^{-11}	7.15×10^{-9}
GO:0005516~calmodulin binding	19	6.7	5.09×10^{-10}	2.81×10^{-7}
GO:0003779~actin binding	25	8.8	6.35×10^{-10}	3.51×10^{-7}

Table 6: Top 10 GO, BP, CC, and MF pathways associated with proteins that have amphetamine-dependent increases in spinophilin. The 286 proteins that had an increased association with spinophilin (log 2 ratio ≥ 0.05) were input into the DAVID Bioinformatics resource and analyzed using GO BP, CC, and MF pathway analyses. The top 10 enriched pathways are shown. Adopted/Modified from (Watkins et al., 2018). Created with BioRender.com.

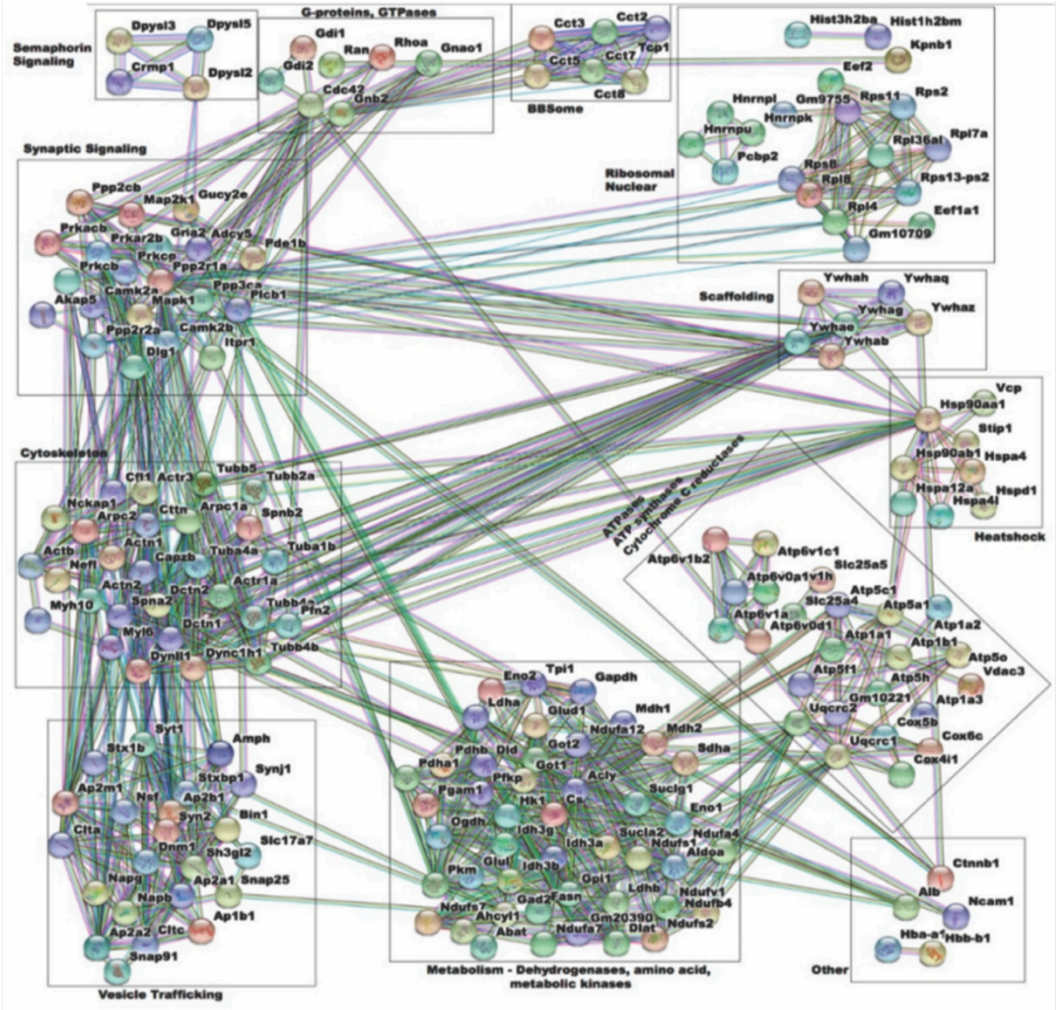

Figure 25: Graphical Representation of Spinophilin Interactors. Proteins with greater abundance in HA immunoprecipitates isolated from amphetamine-treated animals were input into the string-db program (www.string-db.org) and separated by hand based on function. To reduce the complexity of this map, only those proteins that had at least 1 interaction at a confidence of 0.9 (highest confidence). Adopted/Modified from (Watkins et al., 2018). Created with BioRender.com.

Alteration and Validation of Novel Spinophilin Interacting Proteins

While some of the proteins isolated using the HA spinophilin antibody are known spinophilin interactors (e.g., PP1, glutamate receptors, actin), some have not been previously validated. Therefore, we wanted to validate some of the proteins that had an altered association with spinophilin that are involved in different processes. We chose SAP102 (*Dlg3*), src kinase inhibitor protein 1 (*Srcin1*), and clathrin heavy chain (*Cltc*) as proteins involved in synaptic scaffolding, signaling, and vesicle trafficking, respectively. To determine if these proteins interact with spinophilin in a specific manner, we dissected out total (dorsal and ventral (e.g., accumbens)) striatum (Str) and olfactory tubercle (OT), a further ventral portion of the striatum from wildtype and whole-body spinophilin KO animals. We chose these regions based on their roles in psychostimulant sensitization (Durieux et al., 2009; Flanigan & LeClair, 2017; Ikemoto, 2007). We immunoprecipitated striatal and tubercle lysates with a spinophilin antibody and immunoblotted for spinophilin, SAP102, Srcin1, and clathrin heavy chain. Spinophilin was only detected in WT and not KO samples (**Figure 26**). Moreover, spinophilin interactions were only detected in WT and not KO animals. These data suggest that these proteins are specific interactors with spinophilin. Future studies will be needed to fully validate that the amphetamine-dependent increased association with spinophilin is direct protein-protein interactions.

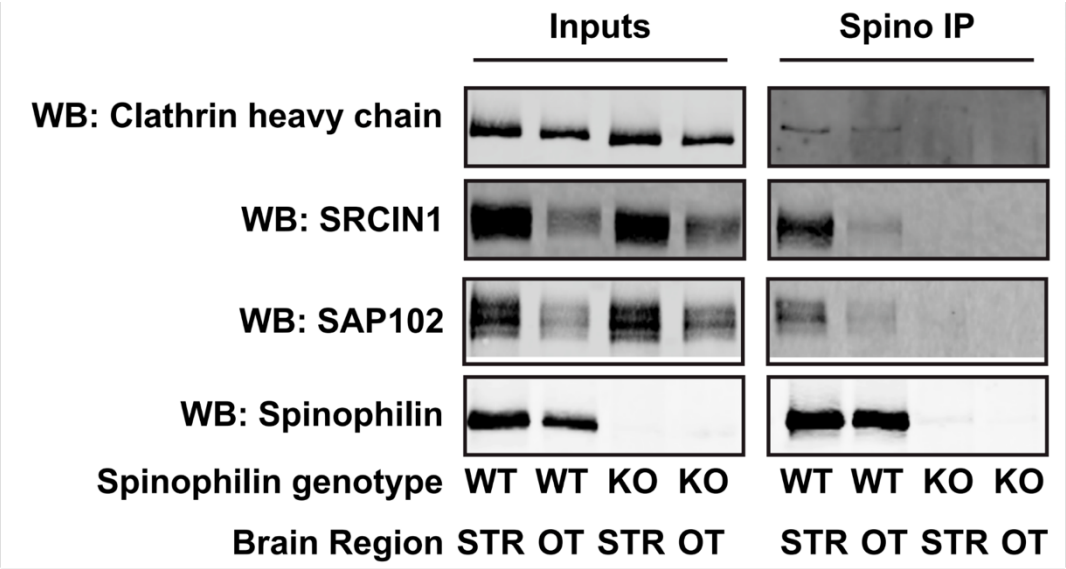

Figure 26: Validation of spinophilin interactions. WT or spinophilin KO striatal (STR) or olfactory tubercle (OT) lysates were immunoprecipitated with a spinophilin antibody. Lysates or immunoprecipitates were immunoblotted for spinophilin and three interacting proteins that were detected in the HA immunoprecipitates that had a decreased (SAP102) or increased (Clathrin heavy chain and SRCIN1) interaction with spinophilin in amphetamine-treated animals. Spinophilin and all associated proteins were detected in the spinophilin immunoprecipitates from WT animals but were absent in immunoprecipitates isolated from KO animals. Adopted/Modified from (Watkins et al., 2018). Created with BioRender.com.

Loss of Spinophilin Mediates Amphetamine Dependent Expression Changes in Myriad Post-Synaptic Proteins

We next wanted to see if synaptic proteins critical for proper striatal signaling, and synaptic plasticity, would be affected by a sensitizing amphetamine regimen in spinophilin KO animals. Indeed, loss of spinophilin mediates amphetamine-dependent protein expression and phosphorylated protein expression in myriad synaptic proteins (**Figure 27** and **Figure 28**). Loss of spinophilin prevented amphetamine-dependent increases in GluA1 (significant treatment effect ($F(1, 49) = 5.3$; $P=0.0256$) (**Figure 27E**)), GluA2 (significant interaction effect ($F(1, 49) = 7.6$; $P=0.0081$) (**Figure 27H**)), neurabin (significant interaction effect ($F(1, 46) = 11.71$; $P=0.0013$)) (significant genotype effect ($F(1, 46) = 15.32$; $P=0.0003$)) (**Figure 28C**), SAP102 (significant interaction effect ($F(1, 38) = 13.69$; $P=0.0007$)) (significant genotype effect ($F(1,38) = 5.732$; $P=0.0217$)) (**Figure 28D**) and CaMKIIα (significant interaction effect ($F(1, 30) = 7.719$; $P=0.0093$)) (significant treatment effect ($F(1, 30) = 4.201$; $P=0.0492$) (**Figure 28H**). Amphetamine restores protein expression of GluN2A (significant treatment effect ($F(1, 37) = 9$; $P=0.0046$)) (**Figure 27B**), PSD-95 (significant interaction effect ($F(1, 44) = 23.50$; $P<0.0001$)) (**Figure 28E**) and DARPP-32 (significant treatment effect ($F(1, 30) = 11.71$; $P=0.0018$)) (**Figure 28F**) in spinophilin KOs like saline treated WT levels. Interestingly, loss of spinophilin prevented amphetamine-dependent expression decreases in GluA1 S845 (significant genotype effect ($F(1, 50) = 5.5$; $P=0.0229$)) (significant treatment effect ($F(1, 50) = 4.2$; $P=0.0449$)) (**Figure 27G**). However, saline treated spinophilin KOs were significantly lower than the saline treated WTs.

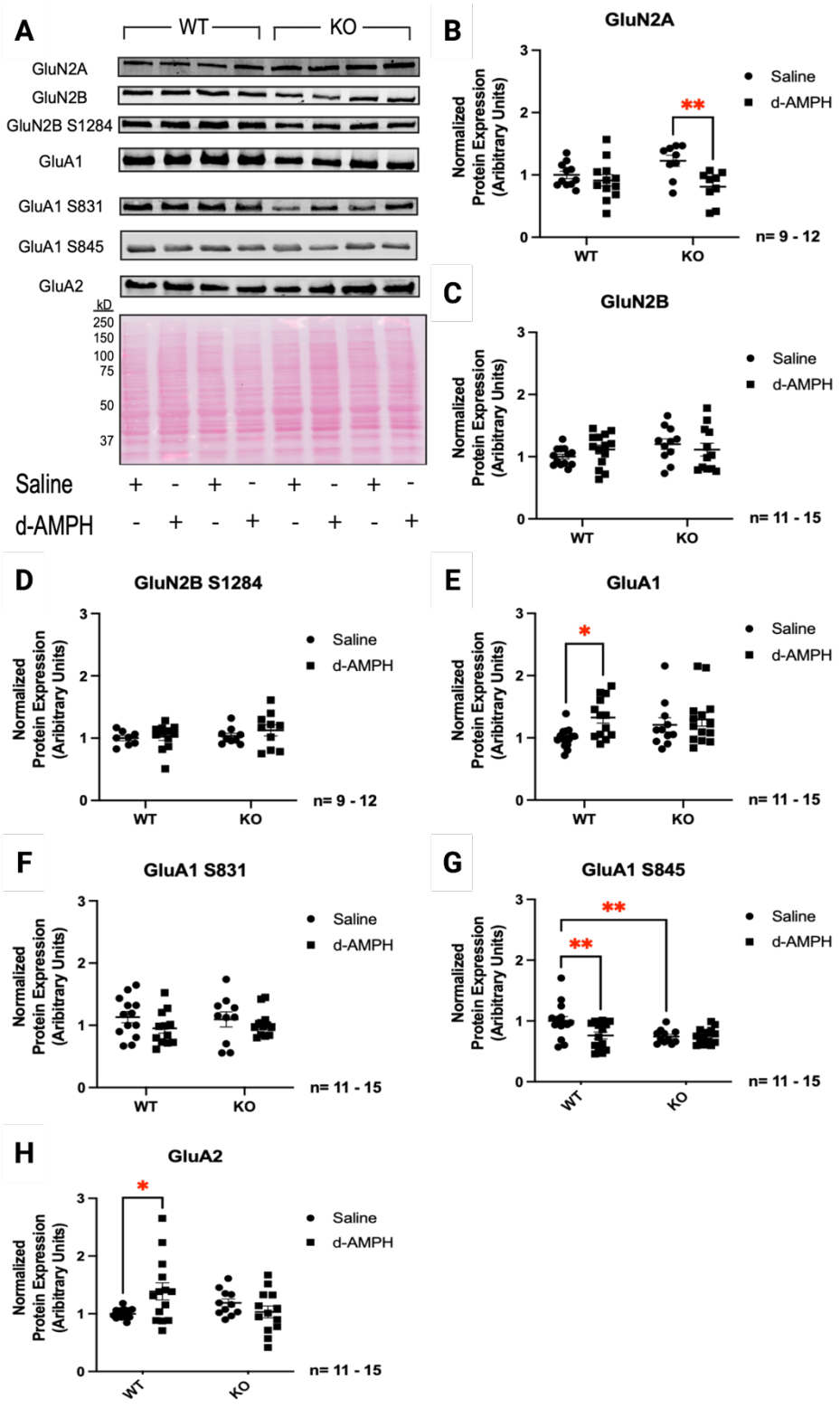

Figure 27: Effects of Amphetamine on Synaptic Proteins in Spinophilin KO. (A) Representative ponceau and immunoblot of NMDAR and AMPAR subunits. (B)

GluN2A- significant treatment effect on KOs (F (1, 37) = 9; P=0.0046. (C) GluN2B- no significance (D) GluN2B S1284- no significance I GluA1- significant treatment effect (F (1, 49) = 5.3; P=0.0256) (F) GluA1 S831- no significance (G) GluA1 S845- significant genotype effect (F (1, 50) = 5.5; P=0.0229), and significant treatment effect (F (1, 50) = 4.2; P=0.0449) (H) GluA2- significant interaction effect (F (1, 49) = 7.6; P=0.0081). All values represent mean ± SEM. Two-way ANOVA with Repeated Measures followed by Tukey or Šídák's *post-hoc*, n= 9-15 per group. *p< 0.05, **p< 0.01 Created with BioRender.com.

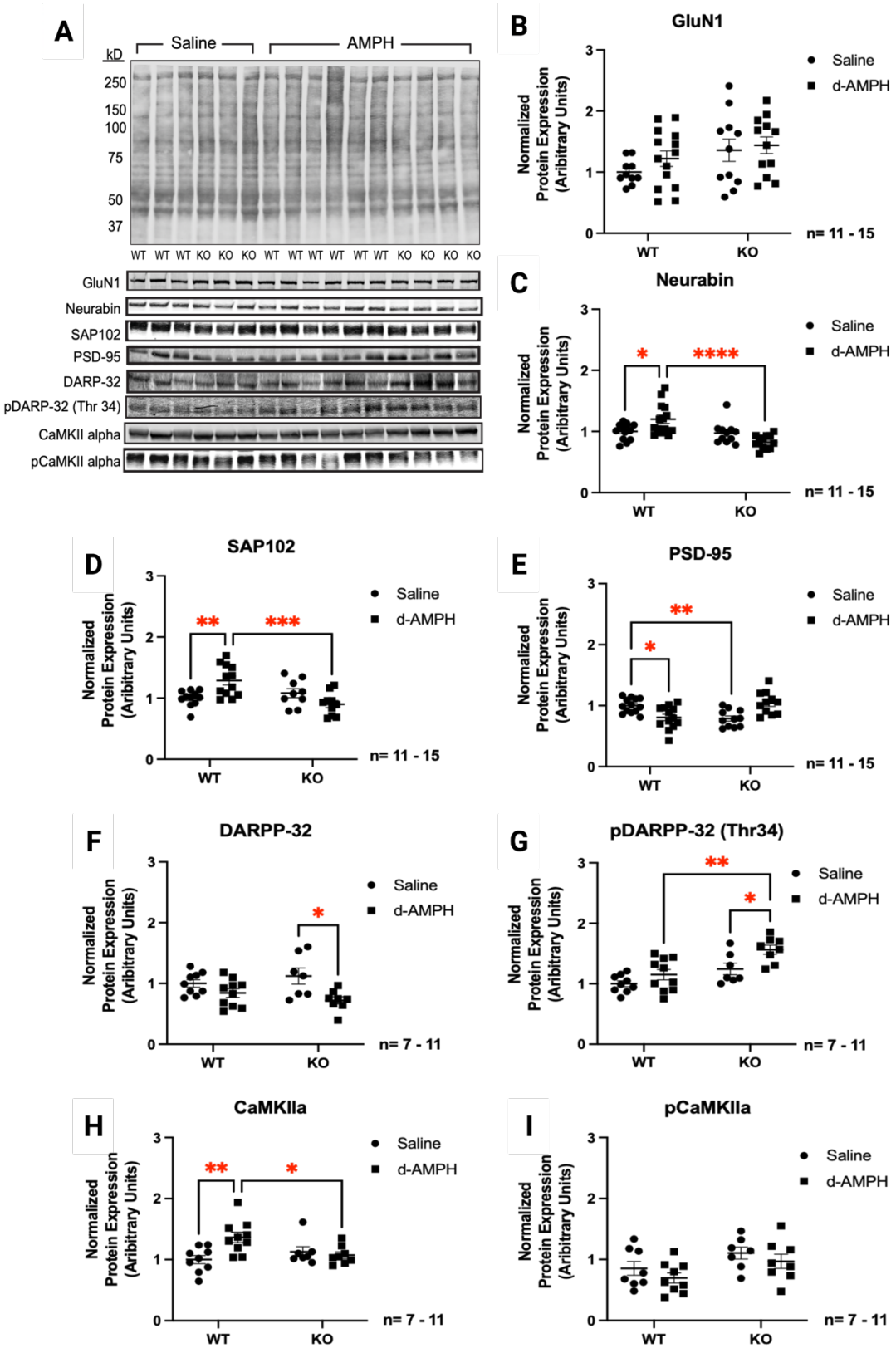
A
Saline
AMPH
kD
250
150
100
75
50
37
WT WT WT KO KO KO WT WT WT WT WT WT KO KO KO KO
GluN1
Neurabin
SAP102
PSD-95
DARP-32
pDARP-32 (Thr 34)
CaMKII alpha
pCaMKII alpha
B
GluN1
Normalized Protein Expression (Aribitrary Units)
Saline
d-AMPH
n= 11 - 15
WT
KO
C
Neurabin
*
****
n= 11 - 15
D
SAP102
**
***
n= 11 - 15
E
PSD-95
**
*
n= 11 - 15
F
DARPP-32
*
n= 7 - 11
G
pDARPP-32 (Thr34)
**
*
n= 7 - 11
H
CaMKIIa
**
*
n= 7 - 11
I
pCaMKIIa
n= 7 - 11

Figure 28: Effects of Amphetamine on Synaptic Proteins in Spinophilin KO. (A) Representative revert-stain and immunoblot of synaptic proteins. (B) GluN1- no significance. (C) Neurabin- significant interaction effect ($F (1, 46) = 11.71$; $P=0.0013$), and a significant genotype effect ($F (1, 46) = 15.32$; $P=0.0003$) (D) SAP102- significant interaction effect ($F (1, 38) = 13.69$; $P=0.0007$ and a significant genotype effect ($F (1,38) = 5.732$; $P=0.0217$) (E) PSD-95- significant interaction effect ($F (1, 44) = 23.50$; $P<0.0001$) (F) DARPP-32- significant treatment effect ($F (1, 30) = 11.71$; $P=0.0018$) (G) pDARPP-32 (Thr 34)- significant genotype effect ($F (1, 30) = 17.91$; $P=0.0002$) and significant treatment effect ($F (1, 30) = 9.075$; $P=0.0052$) (H) CaMKIIα- significant interaction effect ($F (1, 30) = 7.719$; $P=0.0093$) and a significant treatment effect ($F (1, 30) = 4.201$; $P=0.0492$. (I) p CaMKIIα- no significance. All values represent mean ± SEM. Two-way ANOVA with Repeated Measures followed by Tukey or Šídák's *post-hoc*, n= 9-15 per group. *p< 0.05, **p< 0.01, ***p< 0.001, ****p< 0.0001 Created with BioRender.com.

Furthermore, both the saline and amphetamine treated had similar protein expression levels in GluA1 S845 (**Figure 27G**). In addition, phosphorylated DARPP-32 at the Thr34 site (pDARPP-32 (Thr 34)) expression was in enhanced in amphetamine treated spinophilin KOs (significant genotype effect ($F(1, 30) = 17.91$; $P=0.0002$)) (significant treatment effect ($F(1, 30) = 9.075$; $P=0.0052$)) (**Figure 28G**). Interesting, pDARPP-32 in saline treated spinophilin KOs were like WT amphetamine treated animals. Taken together, loss of spinophilin changes the expression of critical post-synaptic proteins that may give rise to the aberrant regulation of striatal signaling and mediated behaviors.

4.4 Discussion

Spinophilin Functional Localization

Spinophilin is a highly abundant spine-enriched protein. We have previously found that loss of spinophilin modulates striatal behaviors. Specifically, loss of spinophilin decreases motor performance and motor learning on a rotarod apparatus (Edler et al., 2018). Moreover, we previously reported that spinophilin KO mice, in contrast to WT mice, do not undergo amphetamine-induced locomotor sensitization (Morris et al., 2018). In addition, others have observed alterations in the response of spinophilin KO mice in rotarod behaviors and following cocaine treatment (Allen et al., 2006). Together, these data suggest that spinophilin is important in striatal based behaviors. However, how spinophilin contributes to these behaviors is unclear. As the major postsynaptic density-enriched PP1-interacting protein, spinophilin's functional regulation of the above striatal behaviors may be due to its targeting of PP1 to synaptic

proteins such as glutamatergic receptor subunits to alter striatal signaling. Indeed, loss of spinophilin drives aberrant changes in glutamatergic subunits, such as GluA1 and GluN2A, AMAPR and NMDAR subunits, respectively, within the striatum (**Figure 27**). Interestingly, GluN2A is a major target for α-synuclein (*SNCA*), which can modulate synaptic transmission in the striatum by targeting GluN2A and α-synuclein was revealed as a spinophilin interacting protein (Durante et al., 2019). Furthermore, spinophilin regulates the phosphorylation of GluA1 S845 during dopaminergic modulation as evident in our western blot studies (**Figure 27**). The phosphorylation status of spinophilin modulates its interaction with actin and the modulation of spinophilin-actin interactions determines where the spinophilin-PP1 complex is anchored within the spine, modifying synaptic signaling, and dendritic spine morphology (Futter et al., 2005; Grossman et al., 2004; Hsieh-Wilson et al., 2003). Moreover, while spinophilin, as its name implies, is enriched in dendritic spines, it is also present in dendrites, presynaptic terminals, and glial cells (E. Chris Muly et al., 2004; E. C. Muly et al., 2004). Therefore, spinophilin may have functions beyond just dendritic spines.

Spinophilin Mediates Striatal Molecular Signaling Adaptations Following Amphetamine Sensitization

To begin to identify cell-type specific spinophilin interactions that may be important in behavioral changes observed following psychostimulant abuse, we created mice that Cre-dependently overexpress an epitope-tagged (HA) form of human spinophilin. Using HA immunoprecipitation from different cell types and TMT labeling, we probed the spinophilin interactome in the striatum of saline compared to amphetamine

treated animals. As previously observed in rats (Boikess & Marshall, 2008; Boikess et al., 2010), the amount of spinophilin detected (based on labeled peptide abundance) was increased following amphetamine treatment. Interestingly, across all samples the abundance of the different proteins was greater in the amphetamine-treated compared to the control treated lysates, even when normalizing to the increased spinophilin expression. This contrasts with what we observed previously in DA-depleted striatum (an animal model of Parkinson disease) (Hiday et al., 2017). In that previous study, 60 proteins were decreased whereas 31 total proteins were increased across two different fractions. In contrast, in the current study, we only observed three proteins that were decreased and 286 proteins that were increased following amphetamine treatment. These data suggest that spinophilin interactions are decreased by DA depletion and increased by hyperdopaminergic signaling. However, it is critical to note that while specific, there are low levels of expression of the HA-tagged, human spinophilin and while we validated expression of the HA-tagged form of the human protein by WB and MS/MS, as well as the interactor, PP1, additional interacting proteins may be non-specifically interacting with the beads. Therefore, future studies will need to follow-up on these studies to delineate those interactions that are real and that are modulated by amphetamine.

Regulation of DA leads to alterations in striatal medium spiny neuron spine density. Specifically, loss of DA decreases spine density (Day et al., 2006; Ingham et al., 1989; Ingham et al., 1993) and psychostimulant treatment increases spine density (Lee et al., 2006; Li et al., 2003). Spinophilin is also known to regulate spine density, with acute knockdown of spinophilin decreasing spine density in hippocampal cultures (Evans et al., 2015). Whole-body spinophilin KO animals do not have loss of dendritic spines in

adulthood (and have a paradoxical increase in young animals) (Feng et al., 2000). This lack of an effect may be due to compensatory changes, such as decreases in expression of PP1 (Lu et al., 2010). Furthermore, the dysregulation of PP1 directly impacts glutamatergic transmission in multiple brain regions (Foley et al., 2021). Previous studies have shown that loss of spinophilin attenuates PP1-mediated AMPAR current rundown (Feng et al., 2000). Indeed, our data shows that loss of spinophilin dysregulates AMPAR subunits (GluA1, GluA2) and GluA1 S845 appropriate expression in response to amphetamine sensitization (**Figure 28 E,G-H**). Furthermore, our data shows that loss of spinophilin disrupts normal psychostimulant-induced expression responses in multiple signaling molecules that participate in dendritic spine dynamics (**Figure 29**). Neuronal scaffolding proteins neurabin, PSD-95, and SAP102 expression responses to amphetamine are reversed in spinophilin KOs (**Figure 29C-D**). Interestingly, researchers have shown amphetamine-dependent increases in spinophilin expression (Boikess & Marshall, 2008; Boikess et al., 2010). In addition, our previous data show a lack of amphetamine-dependent locomotor sensitization (Morris et al., 2018). Taken together with the proteomics data presented, changes in spinophilin expression and/or interactions are critical for normal psychostimulant-induced behaviors. However, it is currently unclear if amphetamine-induced increases in spine density are also abrogated or modulated in spinophilin KO mice.

Classes and Specific Spinophilin Protein Interactions are Modulated by Amphetamine

Using GO and KEGG analysis along with hand annotation of altered interactions in string-db we detailed different classes of spinophilin interacting proteins that have an

increased interaction with amphetamine. These protein classes co-purify with spinophilin, including cytoskeletal and vesicle trafficking proteins (Baucum et al., 2010; Hiday et al., 2017; Hsieh-Wilson et al., 2003; Satoh et al., 1998). Moreover, many of the pathways identified associate with protein binding, striatal function and diseases, and synaptic/postsynaptic protein organization. In addition to these known functions of spinophilin and striatum-dependent regulation by amphetamine, we observed novel/less well-characterized spinophilin interactions. Some of these may be non-specific. For instance, myelin sheath was one of the most abundant cellular components. Myelin sheath components may be non-specifically sticky and may not be specific interactions. Indeed, myelin sheath components are associated with the CRAPome (Mellacheruvu et al., 2013), a list of non-specific interactions that may non-specifically co-precipitate. However, even though some of these interactions may be non-specific, these pathways may be regulated by amphetamines. For instance, psychostimulant abuse in humans may be associated with altered neuron myelination (Berman et al., 2008). Therefore, while additional studies will need to detail specific spinophilin interactions, our data may delineate alterations in protein expression following amphetamine treatment.

Another major class of altered spinophilin interacting proteins were vesicle trafficking proteins. For instance, the synaptic vesicle cycle was identified as being enriched in the KEGG pathway and we delineated multiple vesicle trafficking proteins. While not much is known about the role of spinophilin in vesicle trafficking, one study has detailed spinophilin as a regulator of presynaptic vesicle function (Muhammad et al., 2015). We also validated a vesicle trafficking protein, clathrin heavy chain, as a specific interactor with spinophilin. Spinophilin may play a role in vesicle trafficking on

glutamatergic (or dopaminergic) presynaptic terminals or postsynaptic MSNs; however, given the enrichment of endogenous spinophilin in spines and a lack of detected presynaptic vesicle proteins (e.g., syntaxin, SNAP-25, synaptobrevin, etc.), we posit that this role is more postsynaptic; however, we cannot rule out a presynaptic role for spinophilin in striatum.

We observed many metabolic proteins, including lactate and succinate dehydrogenases, and ATPases, that were increased in spinophilin immunoprecipitates following amphetamine treatment. Moreover, some of the pathways with altered protein expression included glycolytic process, ATP metabolism/hydrolysis/synthesis, tricarboxylic acid cycle, and Citrate (TCA) cycle. Spinophilin has been shown to associate with and regulate the membrane localization of the Na^+-K^+-ATPase (Kimura et al., 2007). Moreover, amphetamines may modify the activity of the Kreb's cycle (King et al., 1975; Valvassori et al., 2013). However, how alterations in spinophilin interactions with metabolic proteins modulate response to amphetamines is an unexplored area.

Direct and Indirect Pathway Striatal MSNs and Spinophilin Interactions

By using mice expressing spinophilin Cre dependently, we were able to isolate complexes from dMSNs and iMSNs. We used a D1 DA receptor Cre line and an A2A adenosine receptor Cre line. These Cre lines were created as part of the GENSAT project (Gerfen et al., 2013; Gong et al., 2007). While D1 and A2A are enriched in striatal MSNs, there may be expression in other cell types and other brain regions. We observed some HA spinophilin expression in other brain regions, including the hippocampus and

prefrontal cortex (data not shown). While these Cre lines only minimally express in these other regions, it may be sufficient for driving expression of the HA spinophilin.

Previous studies have observed persistent psychostimulant-dependent increases in spinophilin density in dMSNs (months), whereas in iMSNs these changes are more transient (days) (Lee et al., 2006). We found that there was a greater association of spinophilin with multiple proteins 72 h following amphetamine treatment and that this occurred in both Cre-driver lines. This suggests that amphetamine may be regulating interactions in both cell types. While our preliminary study suggests the level of increase was greater overall in the dMSNs compared to the iMSNs, it is unclear if these changes will persist in both populations and future studies will need to evaluate the persistence of these changes and the link between spinophilin and modulation of dendritic spine density.

While most protein interaction changes were similar in the two cell types, the magnitude of the effect was different between the different cell types. However, there was a cell-specific effect of amphetamine treatment in two proteins that are known to be involved in striatal pathologies. We detected both α-synuclein and tau (*MAPT*) proteins in the HA-spinophilin immunoprecipitates. While additional work needs to determine if endogenous spinophilin associates with these proteins, it was interesting that amphetamine increased the association of spinophilin with both of these proteins, but this increase was only in the D1 Cre containing animals. These proteins were enriched 2.45-fold (α-synuclein) and 1.85-fold (Tau) in the dMSNs compared to the iMSNs. These proteins play major roles in PD and AD, respectively, and amphetamine is known to increase α-synuclein and tau protein levels (Klongpanichapak et al., 2007; Straiko et al., 2007). In addition, our data shows that amphetamine significantly decreases expression

of GluN2A (a target for α-synuclein) in spinophilin KOs (Durante et al., 2019). Moreover, phosphorylation of these proteins is important in modulating their function and aggregation potential, but if spinophilin plays a role in modulating this aggregation has, to our knowledge, not been evaluated.

4.5 Conclusion

Our data identify novel putative spinophilin interactions that are modulated by amphetamine using proteomics and several critical synaptic proteins that are affected by loss of spinophilin using immunoblotting methodology. Amphetamine increased spinophilin expression and enhanced spinophilin interaction in our proteomics data. Furthermore, loss of spinophilin affected myriad synaptic proteins that play a critical role in striatal function and revealed some of the neuroadaptations involved in amphetamine sensitization. While these changes may occur in both MSN cell types, this preliminary proteomics study suggests dMSNs appear to be more influenced by amphetamine. Indeed, in the western-blot data synaptic proteins such as CaMKII and phosphorylated DARPP-32 were affected by loss of spinophilin and are highly regulated in dMSNs following psychostimulant administration within the striatum. Future studies need to validate these interactions directly with the use of additional approaches that enhance cell-specific expression and interactions (e.g., viral transduction of Cre-dependent epitope tagged spinophilin or Cre-dependently epitope tag the endogenous locus of spinophilin). In addition, the evaluation of long-term amphetamine changes (e.g., 1-month) in the different striatal MSN subtypes as the immunoblot data show dysregulation of synaptic proteins that play a role in dendritic spine density. However, the current proteomics study

is the first to outline potential pathways of spinophilin interactors that are modulated by amphetamine. In addition, the whole-body spinophilin KO data further corroborate aberrant changes in expression of proteins that were revealed in the proteomics data, but also novel synaptic proteins that may be indirectly regulated by spinophilin. Moreover, we have begun to uncover differences in amphetamine-dependent spinophilin interactions in the different striatal cell types and signaling pathways that are affected. This knowledge will enhance our understanding of amphetamine-dependent regulation of cell-specific striatal biology.

Chapter V: Dissertation Summary

5. 1 Summary of Findings

Whole-body spinophilin KO mice exhibit deficits in rotarod motor performance under sedation paradigms (Lu et al., 2010; Wang et al., 2004). In addition, spinophilin KO mice show an enhanced response to psychostimulant-induced conditioned place preference (Allen et al., 2006). Interestingly, both behaviors are mediated, in part, by striatal function. Here we show that global loss of spinophilin mediates basal performance deficits in striatal-mediated rotarod task (**Figure 12**) (Edler et al., 2018). Furthermore, we demonstrate that whole body spinophilin KO mice do not undergo amphetamine-induced sensitization (**Figure 16**), which has been recapitulated using another sensitizing psychostimulant regimen (cocaine) by Areal et al. (Areal et al., 2019; Morris et al., 2018). Taken together, these data suggest that global loss of spinophilin regulates striatal-mediated motor behaviors.

Global loss of spinophilin does not indicate whether the motor perturbations are regulated in a cell-specific manner within the striatum. To address if the deficits observed in the spinophilin global KO animals is cell-specific. We created a conditional spinophilin mouse in which spinophilin is floxed out Cre-dependently (**Figure 9**), we uncovered cell-specific mediation of the rotarod task. More specifically, loss of spinophilin in iMSNs was the major contributor to the deficits found in the rotarod task from the global spinophilin KO mice (**Figure 13**). However, to our surprise, loss of spinophilin in either dMSNs or iMSNs was sufficient to repeat the loss of locomotor

sensitization demonstrated in the global KOs (**Figure 16**, **Figure 17,** and **Figure 19**). Together, the data suggest that MSN cellular subtype-specific loss of spinophilin mediates some tasks (e.g. iMSNs in the rotarod), but loss of spinophilin in either dMSNs or iMSNs mediated another striatal-mediated motor behavior (amphetamine-induced locomotor sensitization).

Previous studies have found that a sensitizing regimen of amphetamine increases spinophilin expression within the striatum (Boikess & Marshall, 2008; Boikess et al., 2010). In addition, striatal dopamine depletion decreases spinophilin's interaction with multiple post-synaptic proteins (Brown et al., 2008; Hiday et al., 2017). Thus, dopaminergic tone and signaling modulates spinophilin function. Using a Cre-dependent HA-epitope tagged spinophilin mouse, we show that not only is spinophilin expression increased following a sensitizing regimen of amphetamine, but that spinophilin expression is increased in both striatal iMSNs and dMSNs (**Figure 22**) (Watkins et al., 2018). Furthermore, we demonstrate that there is increased association with spinophilin and myriad post-synaptic proteins in both iMSNs and dMSNs, in which dMSNs exhibit an overwhelming increase in spinophilin protein-protein interactions (**Figure 23**) (Watkins et al., 2018). Interestingly, using KEGG pathway analysis we uncovered a tripartite convergence within the spinophilin interactome in which kinase and phosphatase proteins were abundantly present between psychostimulant addiction, glutamatergic synapses, and dopaminergic synapses (**Figure 26**). Moreover, global loss of spinophilin dynamically changes the expression and phosphorylation states of some striatal glutamatergic receptors (**Figure 27**). Specifically, whole-body spinophilin KO limits the modulatory impact that amphetamine has on GluA1 and GluA2 expression, in

that there is no longer an increase of these receptor subunits (**Figure 27E, H**). In addition, spinophilin KOs have a basal deficit of phosphorylated GluA1 S845 (a PKA site) (Price et al., 1999; Snyder et al., 2000) that is not downregulated by repeated amphetamine when compared to controls (**Figure 27G**). These changes suggest that loss of spinophilin may limit glutamatergic synaptic transmission through changes in AMPAR subunit expression and phosphorylation under repeated administration of psychostimulants. In addition, global loss of spinophilin dynamically reverses expression of myriad post-synaptic molecules critical for cytoskeleton integrity and PSD organization under an amphetamine-sensitizing regimen (**Figure 28**). Specifically, loss of spinophilin prevents amphetamine-dependent expression changes in synaptic proteins such as neurabin-1, PSD-95, and SAP102, limiting appropriate neuroadaptations that permit amphetamine sensitization (**Figure 28**). Given that spinophilin KOs do not display amphetamine-dependent behavioral sensitization but do show a similar initial response to the drug (Morris et al., 2018), loss of spinophilin may be preventing synaptic re-organization induced by amphetamine that enhances the locomotor response to the psychostimulant over time. Furthermore, spinophilin KOs given repeated amphetamine administration modulate critical striatal signaling proteins like DARPP-32 and CaMKIIα. DARPP-32 expression is decreased in spinophilin KOs treated with amphetamine (**Figure 28**). In addition, phosphorylated Thr34 on DARPP-32 is increased basally in spinophilin KOs and further increased with amphetamine sensitization (**Figure 29F**). Loss of spinophilin prevents amphetamine-dependent increases in CaMKIIα expression (**Figure 28H**). Both DARPP-32 and CaMKIIα are relevant in striatal function and psychostimulant pathology (Bateup et al., 2008; Fernandez et al., 2006; Kourrich et al.,

2012; Loweth et al., 2010; Pizzo et al., 2014; Svenningsson et al., 2005; Yger & Girault, 2011). Taken together spinophilin expression is critical in coordinating and mediating specific phosphorylation and protein expression of striatal synaptic proteins that permit dynamic molecular responsivity to amphetamine-induced sensitization.

5.2 Limitations and Future Directions

A major limitation within my studies is that our animal models do not target cell-specific activity within a specific brain region. Future studies need to be done to reveal distinct mechanisms by which spinophilin directly impacts amphetamine-induced sensitization cell-specifically within the striatum. Here we have uncovered putative target signaling pathways and molecular interactions in which spinophilin may directly or indirectly impact amphetamine-induced sensitization. Furthermore, it is critical to understand that the neuroadaptations that permit sensitization are not fully understood and this research begins to implicate spinophilin function as a prominent player. However, as described below, addressing specific limitations in our study might help elucidate spinophilin's role in amphetamine sensitization and further deepen our understanding of spinophilin function.

Sex as a Biological Variable

An important limitation of these studies is that sex as a biological variable was not considered. There is evidence to suggest that some striatal-mediated motor actions can be altered by estrogen (Meitzen et al., 2018). Specifically, locomotion was increased in

female rodents in which the dorsal striatum was lesioned (Studelska & Beatty, 1978). In addition, increases in rearing were present in dorsal striatum lesioned female rodents that received ovariectomies (Studelska & Beatty, 1978). In addition, MSN electrophysiological properties are influenced by sex and estrogen (Meitzen et al., 2018). Specifically, dorsal striatum MSN firing rates and sensitivity to DA were increased in ovariectomized female rodents that were given estradiol injections (Arnauld et al., 1981). A recent study demonstrated that 17β-estradiol (E2) can increase DA release and DA cellular activity in the striatum (Yoest et al., 2018). In addition, estrogen can increase spinophilin expression (Hao et al., 2003; Lee et al., 2004). We also uncovered that amphetamine increases spinophilin expression and its interaction with myriad associating proteins (Watkins et al., 2018). Together these data may suggest that there could be sex differences in our behavioral studies. However, in our rotarod studies we did not detect any difference between males and females (**Figure 29B-C**), suggesting that either the task is not appropriate to illuminate sex differences in motor skills or simply, sex does not mediate the rotarod task. There was a trend toward reduced sensitization in the spinophilin floxed iMSN males (**Figure 30B**); however, it was not statistically significantly. These data together suggest, that there are no sex differences in the behavioral tasks conducted . However, because we did not sex match our studies such that we could directly compare males and females and in addition we did not synchronize estrous cycles within our female cohorts, we cannot deduce if there are sex differences, and if so mediated, in part, by hormonal changes in our behavioral tasks.

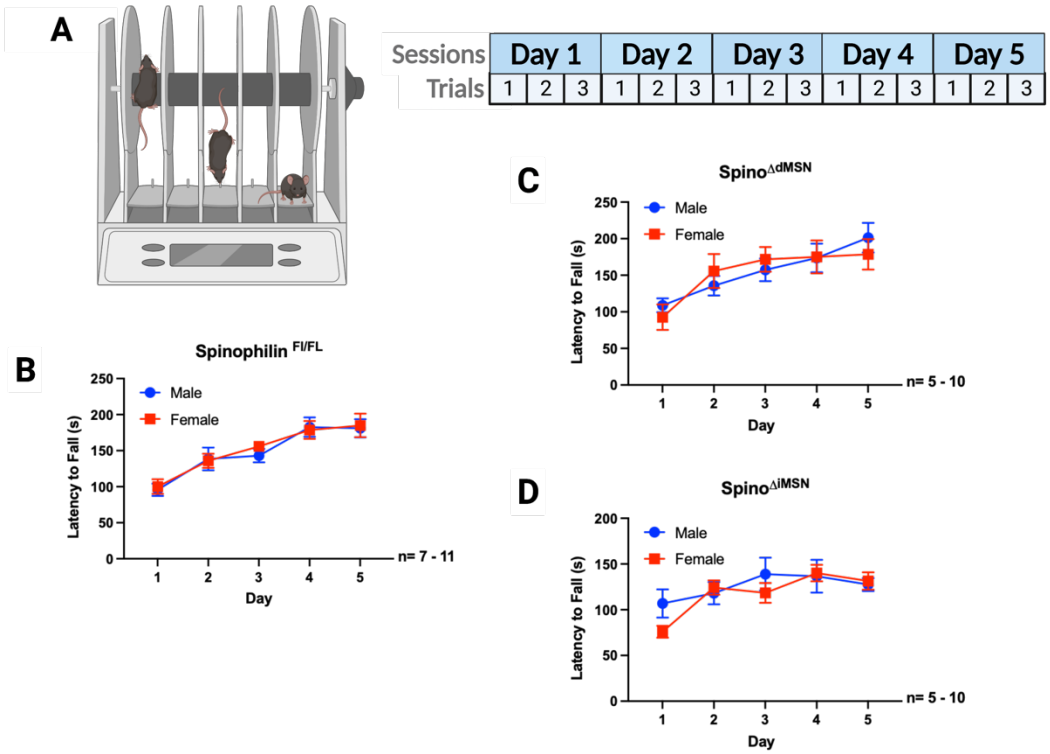

Figure 29: Cell-Specific Motor Skill Learning Deficits Male v Female. (A) Adult (7-8 week old) mice were placed on an accelerating rotarod apparatus. Three trials per day for five consecutive days were performed, and the latency to fall was recorded (modified methodology) (Edler et al., 2018). (B-D) No significant sex differences in any group. Created with BioRender.com.

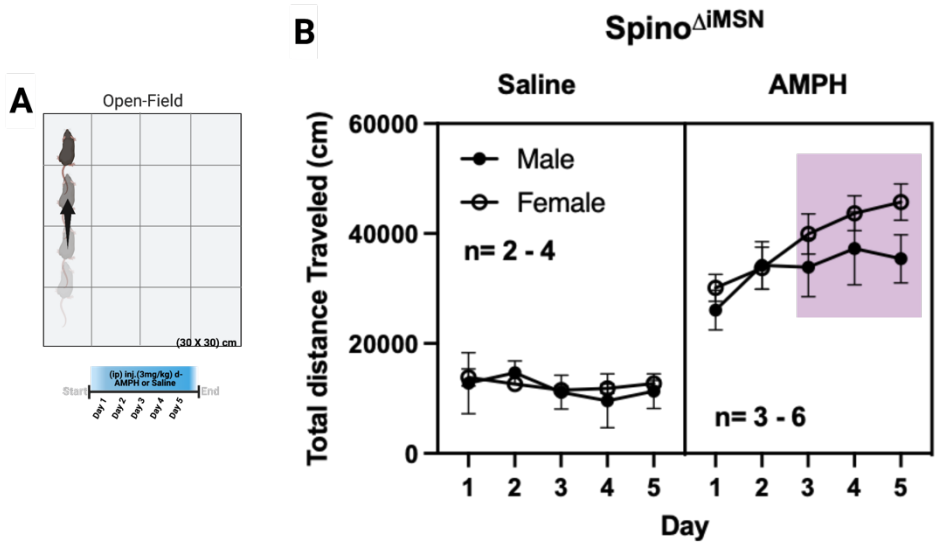

Figure 30: Amphetamine-dependent Behavioral Sensitization in iMSN Spinophilin KO Mice Male v Female. (A) 8-9 week old male and female spino$^{\Delta iMSN}$ along with appropriate control mice underwent an amphetamine-induced behavioral sensitization regimen, as previously described (Morris et al., 2018). Immediately after each injection, mice were placed in a Noldus Phenotyper Cage (30 cm x 30 cm x 30 cm). Each subject's distance travelled was recorded for 1 hour. (B) There were no significant sex differences, but higher standard error of the mean in the amphetamine treated males. Created with BioRender.com.

Striatal Interneuron Motor Control

Parvalbumin and cholinergic interneurons play a critical role in organizing MSN motor-mediated output (Gritton et al., 2019). Specifically, parvalbumin interneurons fine-tune striatal MSN output during motor execution, while cholinergic interneurons amalgamate MSN motor output that signal extinction of motor actions (Gritton et al., 2019). In addition, loss of dopaminergic signaling in cholinergic interneurons ablates cocaine-induced behavioral sensitization (Lewis et al., 2020). Specifically, the activation of D2Rs on cholinergic interneurons inhibit cholinergic activity, thus permitting sustained locomotor activity demonstrated in psychostimulant-induced sensitization (Lewis et al., 2020). Spinophilin binds to D2Rs via its GPCR binding domain (Sarrouilhe et al., 2006; Smith et al., 1999). Furthermore, spinophilin has been shown to be expressed in cortical parvalbumin-containing interneurons (E. Chris Muly et al., 2004). It is possible that spinophilin may be expressed in striatal interneurons. Preliminarily, we show that spinophilin may be expressed in cholinergic interneurons (CINs) using RNAScope (**Figure 31A**). Furthermore, the progeny of spinophilin$^{FL/FL}$ mice crossed with choline acetyltransferase (ChAT)-Cre animals, which presumably deletes spinophilin from cholinergic interneurons within the striatum, unmask a delay in amphetamine-induced behavioral sensitization (**Figure 31B**). These data together may suggest that spinophilin within CINs play a role in amphetamine-induced sensitization and further studies should be done to determine if that is indeed correct. Given that we see, although preliminarily, a delayed response to the initial dose of amphetamine in female rodents containing spinophilin floxed CINs, and the males have a blunted sensitization effect (although not significant) starting around day 3 in the iMSNs, these data together suggest that there

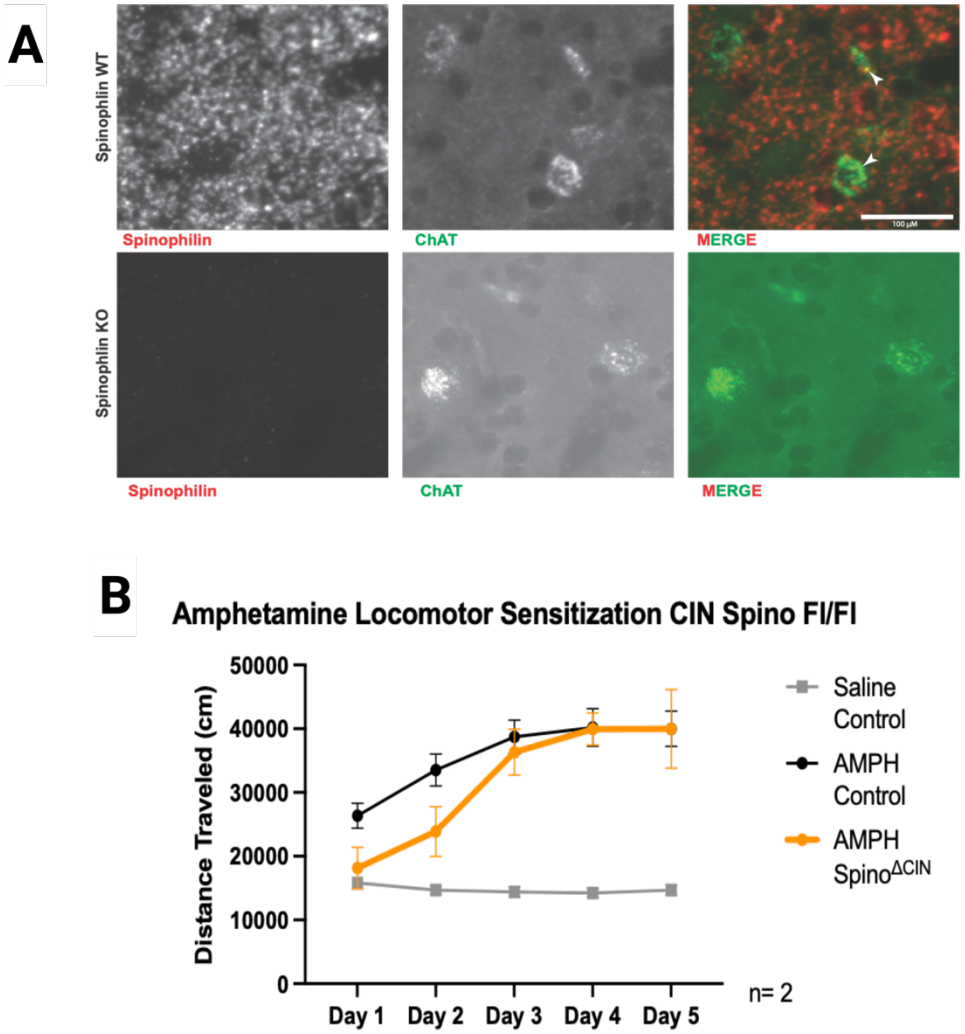

Figure 31: Evidence of Spinophilin in Cholinergic Interneurons. (A) RNAScope showing possible colocalization of spinophilin and ChAT a cholinergic interneuron marker. (B) Loss of spinophilin in cholinergic interneurons causes a delay in amphetamine-induced sensitization. Created with BioRender.com.

may be a sex by cell-type interaction. Interestingly, both cell-types (iMSNs and CINs) contain D2Rs. Ultimately, understanding how spinophilin interacts specifically with D2Rs may uncover spinophilin's role in psychostimulant-induced behavioral sensitization.

Alterations in Structural Plasticity

The global western-blot data together with the cell-specific proteomic data also suggest perturbations in molecular reorganization of striatal synapses during amphetamine sensitization. Indeed, the expression of critical structural synaptic proteins such as PSD-95, SAP-102, and neurabin-1 did not undergo the appropriate amphetamine-dependent neuroadaptations associated with amphetamine sensitization (**Figure 28**). In addition, many NMDAR and AMPAR subunits were also resistant to amphetamine dependent changes (**Figure 27,** and **Figure 28**). Furthermore, our proteomics data revealed that myriad proteins associated with spinophilin are generally upregulated in response to amphetamine sensitization (**Figure 22D** and **Figure 23A-B**). Given that the typical response for many spinophilin-associated proteins is an upregulation in expression and association, while loss of spinophilin produced a more rigid molecular response in protein expression of several proteins associated with structural change, suggesting that spinophilin is critical in facilitating amphetamine-dependent neuroadaptations. Psychostimulants are known to induce structural alterations to dendritic spine density and morphology within the striatum. Specifically, repeated injections of psychostimulants increase dendritic spine density within the striatum (Deng et al., 2010; Jedynak et al., 2007; Lee et al., 2006). Furthermore, these increases can be long-lasting (Lee et al., 2006;

Singer et al., 2009). In addition, repeated exposure to psychostimulants increases F-actin, a critical protein for dendritic spine dynamics (Toda et al., 2006). Moreover, spinophilin binds actin directly which is essential to target spinophilin to other substrates within the dendritic spine (Grossman et al., 2002; Satoh et al., 1998). It is plausible that the global spinophilin KOs did not undergo amphetamine-induced behavioral sensitization due to the inability to adapt to synaptic structural changes to repeated amphetamine exposure. Taken together, although our studies do not directly address if loss of spinophilin mediates structural inflexibility, our data does suggest that loss of spinophilin prevents amphetamine-dependent protein expression neuroadaptations within the striatum (**Figure 27** and **Figure 28**). To better probe if loss of spinophilin regulates dendritic spine dynamics after repeated amphetamine exposure, an analysis of dendritic spine density and morphology can be done by injecting MSNs with an iontophoretic fluorescent dye (i.e. Lucifer Yellow) following a sensitizing regimen of amphetamine. Then using confocal microscopy and imaging analysis software (i.e. NeuronStudio) the results will reveal if, in fact, loss of spinophilin prevents amphetamine-dependent dendritic spine dynamics, thus contributing to the impediment of amphetamine-dependent behavioral sensitization.

Brain Region Specificity

The brain region of interest in this work was the striatum; however, given that the cell-specific spinophilin floxed animal model did not fully recapitulate any of the global spinophilin KO behavioral data, it is plausible that there are other brain regions that may have contributed to the results displayed. Myriad brain regions express D1 or A2A

receptors on various cell-types. In addition, spinophilin is localized to several brain regions that have D1 and/or A2A receptors. For example, the primary motor cortex (M1) and the prefrontal cortex also express D1-and D2-containing neurons, and the prefrontal cortex also expresses low levels of A2A receptors. Spinophilin is expressed in both the motor cortex, and prefrontal cortex (Carretón et al., 2012; E. C. Muly et al., 2004). Moreover, dopamine signaling within M1 optimizes motor skill learning (Molina-Luna et al., 2009; Rioult-Pedotti et al., 2015). Taken together the cell-specific spinophilin floxed model was not limited to just striatal function, but also other critical brain regions that could affect the interpretation of the results. The global rotarod data display a basal performance deficit starting day 1 (**Figure 12**) and although the iMSN spinophilin floxed animals do indeed display rotarod deficits (**Figure 13**), the deficits did not start on day 1 and did not recapitulate the global KOs. It is plausible that loss of spinophilin in M1 also contributed to the deficits demonstrated in the global KO and the A2A spinophilin floxed animals. In addition, psychostimulants affect the prefrontal cortex, as evident by the increase in dendritic spine density after repeated IP injections of amphetamine (Singer et al., 2009). We could not recapitulate the loss of amphetamine-induced behavioral sensitization observed in the global spinophilin KOs with our cell-specific spinophilin floxed model. Taken together, loss of spinophilin may be necessary in both cell-types and in multiple brain regions such as M1, prefrontal cortex and the striatum to reproduce the behavioral sensitization impairment. To address these concerns creating mice in which spinophilin is lost in both dMSNs and iMSN may immediately recapitulate the global KO data. However, to probe if the behavioral results are brain region specific, we could utilize recombinant adenoassociated viral vectors that express Cre recombinase and

deliver via stereotaxic surgery, knocking out spinophilin in a brain region-specific manner. Ultimately, understanding how spinophilin functionally contributes to motor actions such as rotarod and psychostimulant-induced behavioral sensitization can lay the groundwork for novel mechanisms in myriad striatal-mediated disease states.

References

Abdi, A., Mallet, N., Mohamed, F. Y., Sharott, A., Dodson, P. D., Nakamura, K. C., Suri, S., Avery, S. V., Larvin, J. T., Garas, F. N., Garas, S. N., Vinciati, F., Morin, S., Bezard, E., Baufreton, J., & Magill, P. J. (2015). Prototypic and arkypallidal neurons in the dopamine-intact external globus pallidus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *35*(17), 6667-6688. https://doi.org/10.1523/JNEUROSCI.4662-14.2015

Ahmari, S. E., Spellman, T., Douglass, N. L., Kheirbek, M. A., Simpson, H. B., Deisseroth, K., Gordon, J. A., & Hen, R. (2013). Repeated cortico-striatal stimulation generates persistent OCD-like behavior. *Science (New York, N.Y.)*, *340*(6137), 1234-1239. https://doi.org/10.1126/science.1234733

Albin, R. L., Young, A. B., & Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends in Neurosciences*, *12*(10), 366-375. https://doi.org/https://doi.org/10.1016/0166-2236(89)90074-X

Aldridge, J. W., Berridge, K. C., & Rosen, A. R. (2004). Basal ganglia neural mechanisms of natural movement sequences. *Can J Physiol Pharmacol*, *82*(8-9), 732-739. https://doi.org/10.1139/y04-061

Allen, P. B., Ouimet, C. C., & Greengard, P. (1997). Spinophilin, a novel protein phosphatase 1 binding protein localized to dendritic spines. *Proc Natl Acad Sci U S A*, *94*(18), 9956-9961. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9275233

Allen, P. B., Zachariou, V., Svenningsson, P., Lepore, A. C., Centonze, D., Costa, C., Rossi, S., Bender, G., Chen, G., Feng, J., Snyder, G. L., Bernardi, G., Nestler, E. J., Yan, Z., Calabresi, P., & Greengard, P. (2006). Distinct roles for spinophilin and neurabin in dopamine-mediated plasticity. *Neuroscience*, *140*(3), 897-911. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16600521

Allichon, M.-C., Ortiz, V., Pousinha, P., Andrianarivelo, A., Petitbon, A., Heck, N., Trifilieff, P., Barik, J., & Vanhoutte, P. (2021). Cell-Type-Specific Adaptions in Striatal Medium-Sized Spiny Neurons and Their Roles in Behavioral Responses to Drugs of Abuse [Review]. *Frontiers in Synaptic Neuroscience*, *13*. https://doi.org/10.3389/fnsyn.2021.799274

Ardito, F., Giuliani, M., Perrone, D., Troiano, G., & Lo Muzio, L. (2017). The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy (Review). *International journal of molecular medicine*, *40*(2), 271-280. https://doi.org/10.3892/ijmm.2017.3036

Areal, L. B., Hamilton, A., Martins-Silva, C., Pires, R. G. W., & Ferguson, S. S. G. (2019). Neuronal scaffolding protein spinophilin is integral for cocaine-induced behavioral sensitization and ERK1/2 activation. *Molecular Brain*, *12*(1), 15. https://doi.org/10.1186/s13041-019-0434-7

Arnauld, E., Dufy, B., Pestre, M., & Vincent, J. D. (1981). Effects of estrogens on the responses of caudate neurons to microiontophoretically applied dopamine. *Neurosci Lett*, *21*(3), 325-331. https://doi.org/10.1016/0304-3940(81)90225-1

Ashok, A. H., Mizuno, Y., Volkow, N. D., & Howes, O. D. (2017). Association of Stimulant Use With Dopaminergic Alterations in Users of Cocaine, Amphetamine, or Methamphetamine: A Systematic Review and Meta-analysis. *JAMA psychiatry*, *74*(5), 511-519. https://doi.org/10.1001/jamapsychiatry.2017.0135

Asser, A., & Taba, P. (2015). Psychostimulants and movement disorders. *Frontiers in neurology*, *6*, 75-75. https://doi.org/10.3389/fneur.2015.00075

Augustin, S. M., Beeler, J. A., McGehee, D. S., & Zhuang, X. (2014). Cyclic AMP and afferent activity govern bidirectional synaptic plasticity in striatopallidal neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *34*(19), 6692-6699. https://doi.org/10.1523/jneurosci.3906-13.2014

Augustin, S. M., Loewinger, G. C., O'Neal, T. J., Kravitz, A. V., & Lovinger, D. M. (2020). Dopamine D2 receptor signaling on iMSNs is required for initiation and vigor of learned actions. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *45*(12), 2087-2097. https://doi.org/10.1038/s41386-020-00799-1

Ayton, S., George, J. L., Adlard, P. A., Bush, A. I., Cherny, R. A., & Finkelstein, D. I. (2013). The effect of dopamine on MPTP-induced rotarod disability. *Neurosci Lett*, *543*, 105-109. https://doi.org/10.1016/j.neulet.2013.02.066

Báez-Mendoza, R., & Schultz, W. (2013). The role of the striatum in social behavior. *Frontiers in neuroscience*, *7*, 233-233. https://doi.org/10.3389/fnins.2013.00233

Balleine, B. W., & O'Doherty, J. P. (2010). Human and Rodent Homologies in Action Control: Corticostriatal Determinants of Goal-Directed and Habitual Action. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *35*, 48-69.

Barbera, G., Liang, B., Zhang, L., Gerfen, C. R., Culurciello, E., Chen, R., Li, Y., & Lin, D. T. (2016). Spatially Compact Neural Clusters in the Dorsal Striatum Encode Locomotion Relevant Information. *Neuron*, *92*(1), 202-213. https://doi.org/10.1016/j.neuron.2016.08.037

Bateup, H. S., Santini, E., Shen, W., Birnbaum, S., Valjent, E., Surmeier, D. J., Fisone, G., Nestler, E. J., & Greengard, P. (2010). Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc Natl Acad Sci U S A*, *107*(33), 14845-14850. https://doi.org/10.1073/pnas.1009874107

Bateup, H. S., Svenningsson, P., Kuroiwa, M., Gong, S., Nishi, A., Heintz, N., & Greengard, P. (2008). Cell type-specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. *Nature Neuroscience*, *11*(8), 932-939. https://doi.org/10.1038/nn.2153

Baucum, A. J., 2nd, Brown, A. M., & Colbran, R. J. (2013). Differential association of postsynaptic signaling protein complexes in striatum and hippocampus. *Journal of neurochemistry*, *124*(4), 490-501. https://doi.org/10.1111/jnc.12101

Baucum, A. J., 2nd, Strack, S., & Colbran, R. J. (2012). Age-dependent targeting of protein phosphatase 1 to Ca2+/calmodulin-dependent protein kinase II by

spinophilin in mouse striatum. *PloS one*, *7*(2), e31554-e31554. https://doi.org/10.1371/journal.pone.0031554

Baucum, A. J., Jalan-Sakrikar, N., Jiao, Y., Gustin, R. M., Carmody, L. C., Tabb, D. L., Ham, A.-J. L., & Colbran, R. J. (2010). Identification and Validation of Novel Spinophilin-associated Proteins in Rodent Striatum Using an Enhanced ex Vivo Shotgun Proteomics Approach*. *Molecular & Cellular Proteomics*, *9*(6), 1243-1259. https://doi.org/https://doi.org/10.1074/mcp.M900387-MCP200

Bear, M. F., & Malenka, R. C. (1994). Synaptic plasticity: LTP and LTD. *Current opinion in neurobiology*, *4*(3), 389-399. https://doi.org/https://doi.org/10.1016/0959-4388(94)90101-5

Beaulieu, J. M., Sotnikova, T. D., Gainetdinov, R. R., & Caron, M. G. (2006). Paradoxical striatal cellular signaling responses to psychostimulants in hyperactive mice. *J Biol Chem*, *281*(43), 32072-32080. https://doi.org/10.1074/jbc.M606062200

Beglinger, L. J., Langbehn, D. R., Duff, K., Stierman, L., Black, D. W., Nehl, C., Anderson, K., Penziner, E., & Paulsen, J. S. (2007). Probability of obsessive and compulsive symptoms in Huntington's disease. *Biological psychiatry*, *61*(3), 415-418. https://doi.org/10.1016/j.biopsych.2006.04.034

Belin, D., & Everitt, B. J. (2008). Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. *Neuron*, *57*(3), 432-441. https://doi.org/10.1016/j.neuron.2007.12.019

Benazzouz, A., Breit, S., Koudsie, A., Pollak, P., Krack, P., & Benabid, A. L. (2002). Intraoperative microrecordings of the subthalamic nucleus in Parkinson's disease. *Mov Disord*, *17 Suppl 3*, S145-149. https://doi.org/10.1002/mds.10156

Berman, S., O'Neill, J., Fears, S., Bartzokis, G., & London, E. D. (2008). Abuse of amphetamines and structural abnormalities in the brain. *Annals of the New York Academy of Sciences*, *1141*, 195-220. https://doi.org/10.1196/annals.1441.031

Bernard, V., Somogyi, P., & Bolam, J. P. (1997). Cellular, subcellular, and subsynaptic distribution of AMPA-type glutamate receptor subunits in the neostriatum of the rat. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *17*(2), 819-833. https://doi.org/10.1523/JNEUROSCI.17-02-00819.1997

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

Berridge, K. C., & Aldridge, J. W. (2000). Super-stereotypy I: enhancement of a complex movement sequence by systemic dopamine D1 agonists. *Synapse*, *37*(3), 194-204. https://doi.org/10.1002/1098-2396(20000901)37:3<194::Aid-syn3>3.0.Co;2-a

Berridge, K. C., Aldridge, J. W., Houchard, K. R., & Zhuang, X. (2005). Sequential super-stereotypy of an instinctive fixed action pattern in hyper-dopaminergic mutant mice: a model of obsessive compulsive disorder and Tourette's. *BMC biology*, *3*, 4-4. https://doi.org/10.1186/1741-7007-3-4

Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamales, M., Hervé, D., Valjent, E., & Girault, J.-A. (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and

haloperidol. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *28*(22), 5671-5685. https://doi.org/10.1523/JNEUROSCI.1039-08.2008

Boehm, S. L., 2nd, Moore, E. M., Walsh, C. D., Gross, C. D., Cavelli, A. M., Gigante, E., & Linsenbardt, D. N. (2008). Using drinking in the dark to model prenatal binge-like exposure to ethanol in C57BL/6J mice. *Developmental psychobiology*, *50*(6), 566-578. https://doi.org/10.1002/dev.20320

Boikess, S. R., & Marshall, J. F. (2008). A sensitizing d-amphetamine regimen induces long-lasting spinophilin protein upregulation in the rat striatum and limbic forebrain. *Eur J Neurosci*, *28*(10), 2099-2107. https://doi.org/10.1111/j.1460-9568.2008.06481.x

Boikess, S. R., O'Dell, S. J., & Marshall, J. F. (2010). A sensitizing D-amphetamine dose regimen induces long-lasting spinophilin and VGLUT1 protein upregulation in the rat diencephalon. *Neurosci Lett*, *469*(1), 49-54. https://doi.org/10.1016/j.neulet.2009.11.042

Bollen, M., Peti, W., Ragusa, M. J., & Beullens, M. (2010). The extended PP1 toolkit: designed to create specificity. *Trends Biochem Sci*, *35*(8), 450-458. https://doi.org/10.1016/j.tibs.2010.03.002

Bostan, A. C., & Strick, P. L. (2018). The basal ganglia and the cerebellum: nodes in an integrated network. *Nature Reviews Neuroscience*, *19*(6), 338-350. https://doi.org/10.1038/s41583-018-0002-7

Brown, A. M., Baucum, A. J., Bass, M. A., & Colbran, R. J. (2008). Association of protein phosphatase 1 gamma 1 with spinophilin suppresses phosphatase activity in a Parkinson disease model. *J Biol Chem*, *283*(21), 14286-14294. https://doi.org/10.1074/jbc.M801377200

Burguière, E., Monteiro, P., Mallet, L., Feng, G., & Graybiel, A. M. (2015). Striatal circuits, habits, and implications for obsessive-compulsive disorder. *Current opinion in neurobiology*, *30*, 59-65. https://doi.org/10.1016/j.conb.2014.08.008

Burke, A. R., & Miczek, K. A. (2014). Stress in adolescence and drugs of abuse in rodent models: Role of dopamine, CRF, and HPA axis [journal article]. *Psychopharmacology*, *231*(8), 1557-1580. https://doi.org/10.1007/s00213-013-3369-1

Burrus, C. J., McKinstry, S. U., Kim, N., Ozlu, M. I., Santoki, A. V., Fang, F. Y., Ma, A., Karadeniz, Y. B., Worthington, A. K., Dragatsis, I., Zeitlin, S., Yin, H. H., & Eroglu, C. (2020). Striatal Projection Neurons Require Huntingtin for Synaptic Connectivity and Survival. *Cell reports*, *30*(3), 642-657.e646. https://doi.org/10.1016/j.celrep.2019.12.069

Burton, A. C., Nakamura, K., & Roesch, M. R. (2015). From ventral-medial to dorsal-lateral striatum: neural correlates of reward-guided decision-making. *Neurobiology of learning and memory*, *117*, 51-59. https://doi.org/10.1016/j.nlm.2014.05.003

Calabresi, P., Picconi, B., Tozzi, A., Ghiglieri, V., & Di Filippo, M. (2014). Direct and indirect pathways of basal ganglia: a critical reappraisal. *Nat Neurosci*, *17*(8), 1022-1030. https://doi.org/10.1038/nn.3743

Calabresi, P., Saiardi, A., Pisani, A., Baik, J.-H., Centonze, D., Mercuri, N. B., Bernardi, G., & Borrelli, E. (1997). Abnormal Synaptic Plasticity in the Striatum of Mice

Lacking Dopamine D2 Receptors. *The Journal of Neuroscience*, *17*(12), 4536. https://doi.org/10.1523/JNEUROSCI.17-12-04536.1997

Campos, F. L., Carvalho, M. M., Cristovão, A. C., Je, G., Baltazar, G., Salgado, A. J., Kim, Y.-S., & Sousa, N. (2013). Rodent models of Parkinson's disease: beyond the motor symptomatology. *Frontiers in Behavioral Neuroscience*, *7*, 175-175. https://doi.org/10.3389/fnbeh.2013.00175

Canales, J. J., & Graybiel, A. M. (2000). Patterns of gene expression and behavior induced by chronic dopamine treatments. *Ann Neurol*, *47*(4 Suppl 1), S53-59.

Cannon, J. F. (2010). Function of protein phosphatase-1, Glc7, in Saccharomyces cerevisiae. *Adv Appl Microbiol*, *73*, 27-59. https://doi.org/10.1016/s0065-2164(10)73002-1

Carnero, A. (2012). Spinophilin: a new tumor suppressor at 17q21. *Curr Mol Med*, *12*(5), 528-535. https://doi.org/10.2174/156652412800619987

Carretón, O., Giralt, A., Torres-Peraza, J. F., Brito, V., Lucas, J. J., Ginés, S., Canals, J. M., & Alberch, J. (2012). Age-dependent decline of motor neocortex but not hippocampal performance in heterozygous BDNF mice correlates with a decrease of cortical PSD-95 but an increase of hippocampal TrkB levels. *Exp Neurol*, *237*(2), 335-345. https://doi.org/10.1016/j.expneurol.2012.06.033

Carvalho, A. L., Duarte, C. B., & Carvalho, A. P. (2000). Regulation of AMPA Receptors by Phosphorylation. *Neurochemical Research*, *25*(9), 1245-1255. https://doi.org/10.1023/A:1007644128886

Cass, W. A., Gerhardt, G. A., Mayfield, R. D., Curella, P., & Zahniser, N. R. (1992). Differences in dopamine clearance and diffusion in rat striatum and nucleus accumbens following systemic cocaine administration. *J Neurochem*, *59*(1), 259-266. https://doi.org/10.1111/j.1471-4159.1992.tb08899.x

Centonze, D., Picconi, B., Baunez, C., Borrelli, E., Pisani, A., Bernardi, G., & Calabresi, P. (2002). Cocaine and amphetamine depress striatal GABAergic synaptic transmission through D2 dopamine receptors. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *26*(2), 164-175. https://doi.org/10.1016/s0893-133x(01)00299-8

Cepeda, C., Buchwald, N. A., & Levine, M. S. (1993). Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(20), 9576-9580. https://doi.org/10.1073/pnas.90.20.9576

Charlton, J. J., Allen, P. B., Psifogeorgou, K., Chakravarty, S., Gomes, I., Neve, R. L., Devi, L. A., Greengard, P., Nestler, E. J., & Zachariou, V. (2008). Multiple actions of spinophilin regulate mu opioid receptor function. *Neuron*, *58*(2), 238-247. https://doi.org/10.1016/j.neuron.2008.02.006

Chen, S.-Y., Lu, K.-M., Ko, H.-A., Huang, T.-H., Hao Janice, H.-J., Yan, Y.-T., Chang Sunny, L.-Y., Evans Sylvia, M., & Liu, F.-C. (2020). Parcellation of the striatal complex into dorsal and ventral districts. *Proceedings of the National Academy of Sciences*, *117*(13), 7418-7429. https://doi.org/10.1073/pnas.1921007117

Chiodi, V., Mallozzi, C., Ferrante, A., Chen, J. F., Lombroso, P. J., Di Stasi, A. M. M., Popoli, P., & Domenici, M. R. (2014). Cocaine-induced changes of synaptic transmission in the striatum are modulated by adenosine A2A receptors and

involve the tyrosine phosphatase STEP. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *39*(3), 569-578. https://doi.org/10.1038/npp.2013.229

Cho, D., Zheng, M., Min, C., Ma, L., Kurose, H., Park, J. H., & Kim, K.-M. (2010). Agonist-induced endocytosis and receptor phosphorylation mediate resensitization of dopamine D(2) receptors. *Molecular endocrinology (Baltimore, Md.)*, *24*(3), 574-586. https://doi.org/10.1210/me.2009-0369

Chu, J., Haynes, R. D., Corbel, S. Y., Li, P., González-González, E., Burg, J. S., Ataie, N. J., Lam, A. J., Cranfill, P. J., Baird, M. A., Davidson, M. W., Ng, H.-L., Garcia, K. C., Contag, C. H., Shen, K., Blau, H. M., & Lin, M. Z. (2014). Non-invasive intravital imaging of cellular differentiation with a bright red-excitable fluorescent protein. *Nature methods*, *11*(5), 572-578. https://doi.org/10.1038/nmeth.2888

Chuhma, N., Mingote, S., Kalmbach, A., Yetnikoff, L., & Rayport, S. (2017). Heterogeneity in Dopamine Neuron Synaptic Actions Across the Striatum and Its Relevance for Schizophrenia. *Biological psychiatry*, *81*(1), 43-51. https://doi.org/10.1016/j.biopsych.2016.07.002

Chun, Y. S., Shima, H., Nagasaki, K., Sugimura, T., & Nagao, M. (1994). PP1 gamma 2, a testis-specific protein-serine/threonine-phosphatase type 1 catalytic subunit, is associated with a protein having high sequence homology with the 78-kDa glucose-regulated protein, a member of the 70-kDa heat shock protein family. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(8), 3319-3323. https://doi.org/10.1073/pnas.91.8.3319

Colbran, R. J., Bass, M. A., McNeill, R. B., Bollen, M., Zhao, S., Wadzinski, B. E., & Strack, S. (1997). Association of brain protein phosphatase 1 with cytoskeletal targeting/regulatory subunits. *J Neurochem*, *69*(3), 920-929. https://doi.org/10.1046/j.1471-4159.1997.69030920.x

Costa, R. M., Cohen, D., & Nicolelis, M. A. (2004). Differential corticostriatal plasticity during fast and slow motor skill learning in mice. *Curr Biol*, *14*(13), 1124-1134. https://doi.org/10.1016/j.cub.2004.06.053

Courtemanche, R., & Cammalleri, A. (2019). Basal Ganglia: Striosomes and the Link between Motivation and Action. *Current Biology*, *29*(2), R62-R65. https://doi.org/https://doi.org/10.1016/j.cub.2018.11.051

Creese, I., Sibley, D. R., Hamblin, M. W., & Leff, S. E. (1983). The Classification of Dopamine Receptors: Relationship to Radioligand Binding. *Annual Review of Neuroscience*, *6*(1), 43-71. https://doi.org/10.1146/annurev.ne.06.030183.000355

Crittenden, J. R., & Graybiel, A. M. (2011). Basal Ganglia disorders associated with imbalances in the striatal striosome and matrix compartments. *Front Neuroanat*, *5*, 59. https://doi.org/10.3389/fnana.2011.00059

Cromwell, H. C., Berridge, K. C., Drago, J., & Levine, M. S. (1998). Action sequencing is impaired in D1A-deficient mutant mice. *Eur J Neurosci*, *10*(7), 2426-2432. https://doi.org/10.1046/j.1460-9568.1998.00250.x

D'Souza, M. S. (2015). Glutamatergic transmission in drug reward: implications for drug addiction [Review]. *Frontiers in Neuroscience*, *9*(404). https://doi.org/10.3389/fnins.2015.00404

Daniela S. Andres, M.D., Ph.D. ,, & Olivier Darbin, Ph.D. (2018). Complex Dynamics in the Basal Ganglia: Health and Disease Beyond the Motor System. *The Journal of*

Neuropsychiatry and Clinical Neurosciences, *30*(2), 101-114. https://doi.org/10.1176/appi.neuropsych.17020039

Day, M., Wang, Z., Ding, J., An, X., Ingham, C. A., Shering, A. F., Wokosin, D., Ilijic, E., Sun, Z., Sampson, A. R., Mugnaini, E., Deutch, A. Y., Sesack, S. R., Arbuthnott, G. W., & Surmeier, D. J. (2006). Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nat Neurosci*, *9*(2), 251-259. https://doi.org/10.1038/nn1632

Degenhardt, L., & Hall, W. (2012). Extent of illicit drug use and dependence, and their contribution to the global burden of disease. *Lancet*, *379*(9810), 55-70. https://doi.org/10.1016/s0140-6736(11)61138-0

Dela Peña, I., Gevorkiana, R., & Shi, W.-X. (2015). Psychostimulants affect dopamine transmission through both dopamine transporter-dependent and independent mechanisms. *European journal of pharmacology*, *764*, 562-570. https://doi.org/10.1016/j.ejphar.2015.07.044

DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends in Neurosciences*, *13*(7), 281-285. https://doi.org/https://doi.org/10.1016/0166-2236(90)90110-V

DeLong, M. R., Alexander, G. E., Georgopoulos, A. P., Crutcher, M. D., Mitchell, S. J., & Richardson, R. T. (1984). Role of basal ganglia in limb movements. *Hum Neurobiol*, *2*(4), 235-244.

Deng, J. V., Rodriguiz, R. M., Hutchinson, A. N., Kim, I. H., Wetsel, W. C., & West, A. E. (2010). MeCP2 in the nucleus accumbens contributes to neural and behavioral responses to psychostimulants. *Nat Neurosci*, *13*(9), 1128-1136. https://doi.org/10.1038/nn.2614

Dhawale, A. K., Wolff, S. B. E., Ko, R., & Ölveczky, B. P. (2021). The basal ganglia control the detailed kinematics of learned motor skills. *Nature Neuroscience*, *24*(9), 1256-1269. https://doi.org/10.1038/s41593-021-00889-3

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(14), 5274-5278. https://doi.org/10.1073/pnas.85.14.5274

Di Sebastiano, A. R., Fahim, S., Dunn, H. A., Walther, C., Ribeiro, F. M., Cregan, S. P., Angers, S., Schmid, S., & Ferguson, S. S. G. (2016). Role of Spinophilin in Group I Metabotropic Glutamate Receptor Endocytosis, Signaling, and Synaptic Plasticity. *The Journal of biological chemistry*, *291*(34), 17602-17615. https://doi.org/10.1074/jbc.M116.722355

Ding, J., Peterson, J. D., & Surmeier, D. J. (2008). Corticostriatal and thalamostriatal synapses have distinctive properties. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *28*(25), 6483-6492. https://doi.org/10.1523/JNEUROSCI.0435-08.2008

Dong, H. W. (2008). *The Allen Reference Atlas: A Digital Color Brain Atlas of C57BL/6J Male Mouse*. John Wiley & Sons.

Doya, K., & Kimura, M. (2014). Chapter 17 - The Basal Ganglia, Reinforcement Learning, and the Encoding of Value. In P. W. Glimcher & E. Fehr (Eds.), *Neuroeconomics (Second Edition)* (pp. 321-333). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-416008-8.00017-6

Durante, V., de Iure, A., Loffredo, V., Vaikath, N., De Risi, M., Paciotti, S., Quiroga-Varela, A., Chiasserini, D., Mellone, M., Mazzocchetti, P., Calabrese, V., Campanelli, F., Mechelli, A., Di Filippo, M., Ghiglieri, V., Picconi, B., El-Agnaf, O. M., De Leonibus, E., Gardoni, F., . . . Calabresi, P. (2019). Alpha-synuclein targets GluN2A NMDA receptor subunit causing striatal synaptic dysfunction and visuospatial memory alteration. *Brain*, *142*(5), 1365-1385. https://doi.org/10.1093/brain/awz065

Durieux, P. F., Bearzatto, B., Guiducci, S., Buch, T., Waisman, A., Zoli, M., Schiffmann, S. N., & de Kerchove d'Exaerde, A. (2009). D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nature Neuroscience*, *12*, 393. https://doi.org/10.1038/nn.2286

https://www.nature.com/articles/nn.2286#supplementary-information

Durieux, P. F., Schiffmann, S. N., & de Kerchove d'Exaerde, A. (2012). Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. *The EMBO journal*, *31*(3), 640-653. https://doi.org/10.1038/emboj.2011.400

Edler, M. C., Salek, A. B., Watkins, D. S., Kaur, H., Morris, C. W., Yamamoto, B. K., & Baucum, A. J., 2nd. (2018). Mechanisms Regulating the Association of Protein Phosphatase 1 with Spinophilin and Neurabin. *ACS chemical neuroscience*, *9*(11), 2701-2712. https://doi.org/10.1021/acschemneuro.8b00144

Emmi, A., Antonini, A., Macchi, V., Porzionato, A., & De Caro, R. (2020). Anatomy and Connectivity of the Subthalamic Nucleus in Humans and Non-human Primates [Review]. *Frontiers in Neuroanatomy*, *14*(13). https://doi.org/10.3389/fnana.2020.00013

Erlendsson, S., Thorsen, T. S., Vauquelin, G., Ammendrup-Johnsen, I., Wirth, V., Martinez, K. L., Teilum, K., Gether, U., & Madsen, K. L. (2019). Mechanisms of PDZ domain scaffold assembly illuminated by use of supported cell membrane sheets. *eLife*, *8*, e39180. https://doi.org/10.7554/eLife.39180

Evans, J. C., Robinson, C. M., Shi, M., & Webb, D. J. (2015). The guanine nucleotide exchange factor (GEF) Asef2 promotes dendritic spine formation via Rac activation and spinophilin-dependent targeting. *The Journal of biological chemistry*, *290*(16), 10295-10308. https://doi.org/10.1074/jbc.M114.605543

Fabbri, M., Reimão, S., Carvalho, M., Nunes, R. G., Abreu, D., Guedes, L. C., Bouça, R., Lobo, P. P., Godinho, C., Coelho, M., Gonçalves, N. C., Rosa, M. M., Antonini, A., & Ferreira, J. J. (2017). Substantia Nigra Neuromelanin as an Imaging Biomarker of Disease Progression in Parkinson's Disease. *J Parkinsons Dis*, *7*(3), 491-501. https://doi.org/10.3233/jpd-171135

Feng, J., Yan, Z., Ferreira, A., Tomizawa, K., Liauw, J. A., Zhuo, M., Allen, P. B., Ouimet, C. C., & Greengard, P. (2000). Spinophilin regulates the formation and function of dendritic spines. *Proc Natl Acad Sci U S A*, *97*(16), 9287-9292. https://doi.org/10.1073/pnas.97.16.9287

Fernandez, E., Schiappa, R., Girault, J.-A., & Le Novère, N. (2006). DARPP-32 is a robust integrator of dopamine and glutamate signals. *PLoS computational biology*, *2*(12), e176-e176. https://doi.org/10.1371/journal.pcbi.0020176

Fernández-García, S., Conde-Berriozabal, S., García-García, E., Gort-Paniello, C., Bernal-Casas, D., García-Díaz Barriga, G., López-Gil, J., Muñoz-Moreno, E.,

Soria, G., Campa, L., Artigas, F., Rodríguez, M. J., Alberch, J., & Masana, M. (2020). M2 cortex-dorsolateral striatum stimulation reverses motor symptoms and synaptic deficits in Huntington's disease. *eLife*, *9*, e57017. https://doi.org/10.7554/eLife.57017

Flanigan, M., & LeClair, K. (2017). Shared Motivational Functions of Ventral Striatum D1 and D2 Medium Spiny Neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *37*(26), 6177-6179. https://doi.org/10.1523/JNEUROSCI.0882-17.2017

Foley, K., McKee, C., Nairn, A. C., & Xia, H. (2021). Regulation of Synaptic Transmission and Plasticity by Protein Phosphatase 1. *The Journal of Neuroscience*, *41*(14), 3040. https://doi.org/10.1523/JNEUROSCI.2026-20.2021

Freeze, B. S., Kravitz, A. V., Hammack, N., Berke, J. D., & Kreitzer, A. C. (2013). Control of basal ganglia output by direct and indirect pathway projection neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *33*(47), 18531-18539. https://doi.org/10.1523/JNEUROSCI.1278-13.2013

Futter, M., Uematsu, K., Bullock Stewart, A., Kim, Y., Hemmings Hugh, C., Nishi, A., Greengard, P., & Nairn Angus, C. (2005). Phosphorylation of spinophilin by ERK and cyclin-dependent PK 5 (Cdk5). *Proceedings of the National Academy of Sciences*, *102*(9), 3489-3494. https://doi.org/10.1073/pnas.0409802102

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

Gerdeman, G. L., Partridge, J. G., Lupica, C. R., & Lovinger, D. M. (2003). It could be habit forming: drugs of abuse and striatal synaptic plasticity. *Trends in Neurosciences*, *26*(4), 184-192. https://doi.org/https://doi.org/10.1016/S0166-2236(03)00065-1

Gerfen, C. R. (1992). The Neostriatal Mosaic: Multiple Levels of Compartmental Organization in the Basal Ganglia. *Annual Review of Neuroscience*, *15*(1), 285-320. https://doi.org/10.1146/annurev.ne.15.030192.001441

Gerfen, C. R., & Bolam, J. P. (2010). The Neuroanatomical Organization of the Basal Ganglia. *Handbook of Behavioral Neuroscience*, *20*, 3-28.

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., Jr., & Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, *250*(4986), 1429-1432. https://doi.org/10.1126/science.2147780

Gerfen, C. R., Paletzki, R., & Heintz, N. (2013). GENSAT BAC cre-recombinase driver lines to study the functional organization of cerebral cortical and basal ganglia circuits. *Neuron*, *80*(6), 1368-1383. https://doi.org/10.1016/j.neuron.2013.10.016

Gerfen, C. R., & Surmeier, D. J. (2011). Modulation of striatal projection systems by dopamine. *Annual Review of Neuroscience*, *34*, 441-466. https://doi.org/10.1146/annurev-neuro-061010-113641

Gerfen, C. R., & Young, W. S., 3rd. (1988). Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in

situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain research*, *460*(1), 161-167. https://doi.org/10.1016/0006-8993(88)91217-6
Gertler, T. S., Chan, C. S., & Surmeier, D. J. (2008). Dichotomous Anatomical Properties of Adult Striatal Medium Spiny Neurons. *The Journal of Neuroscience*, *28*(43), 10814. https://doi.org/10.1523/JNEUROSCI.2660-08.2008
Giordano, N., Iemolo, A., Mancini, M., Cacace, F., De Risi, M., Latagliata, E. C., Ghiglieri, V., Bellenchi, G. C., Puglisi-Allegra, S., Calabresi, P., Picconi, B., & De Leonibus, E. (2018). Motor learning and metaplasticity in striatal neurons: relevance for Parkinson's disease. *Brain*, *141*(2), 505-520. https://doi.org/10.1093/brain/awx351
Gittis, A. H., Berke, J. D., Bevan, M. D., Chan, C. S., Mallet, N., Morrow, M. M., & Schmidt, R. (2014). New Roles for the External Globus Pallidus in Basal Ganglia Circuits and Behavior. *The Journal of Neuroscience*, *34*(46), 15178. https://doi.org/10.1523/JNEUROSCI.3252-14.2014
Gong, S., Doughty, M., Harbaugh, C. R., Cummins, A., Hatten, M. E., Heintz, N., & Gerfen, C. R. (2007). Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *27*(37), 9817-9823. https://doi.org/10.1523/JNEUROSCI.2707-07.2007
Graybiel, A. M. (2008). Habits, rituals, and the evaluative brain. *Annu Rev Neurosci*, *31*, 359-387. https://doi.org/10.1146/annurev.neuro.29.051605.112851
Graybiel, A. M., & Grafton, S. T. (2015). The striatum: where skills and habits meet. *Cold Spring Harbor perspectives in biology*, *7*(8), a021691-a021691. https://doi.org/10.1101/cshperspect.a021691
Greengard, P., Allen, P. B., & Nairn, A. C. (1999). Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron*, *23*(3), 435-447. https://doi.org/10.1016/s0896-6273(00)80798-9
Gremel, C. M., & Lovinger, D. M. (2017). Associative and sensorimotor cortico-basal ganglia circuit roles in effects of abused drugs. *Genes Brain Behav*, *16*(1), 71-85. https://doi.org/10.1111/gbb.12309
Gritton, H. J., Howe, W. M., Romano, M. F., DiFeliceantonio, A. G., Kramer, M. A., Saligrama, V., Bucklin, M. E., Zemel, D., & Han, X. (2019). Unique contributions of parvalbumin and cholinergic interneurons in organizing striatal networks during movement. *Nature Neuroscience*, *22*(4), 586-597. https://doi.org/10.1038/s41593-019-0341-3
Groenewegen, H. J. (2003). The basal ganglia and motor control. *Neural plasticity*, *10*(1-2), 107-120. https://doi.org/10.1155/NP.2003.107
Grossman, S. D., Futter, M., Snyder, G. L., Allen, P. B., Nairn, A. C., Greengard, P., & Hsieh-Wilson, L. C. (2004). Spinophilin is phosphorylated by Ca2+/calmodulin-dependent protein kinase II resulting in regulation of its binding to F-actin. *J Neurochem*, *90*(2), 317-324. https://doi.org/10.1111/j.1471-4159.2004.02491.x
Grossman, S. D., Hsieh-Wilson, L. C., Allen, P. B., Nairn, A. C., & Greengard, P. (2002). The actin-binding domain of spinophilin is necessary and sufficient for targeting to dendritic spines. *Neuromolecular Med*, *2*(1), 61-69. https://doi.org/10.1385/nmm:2:1:61

Hanganu, A., Provost, J.-S., & Monchi, O. (2015). Neuroimaging studies of striatum in cognition part II: Parkinson's disease [Review]. *Frontiers in Systems Neuroscience*, *9*. https://doi.org/10.3389/fnsys.2015.00138

Hao, J., Janssen, W. G., Tang, Y., Roberts, J. A., McKay, H., Lasley, B., Allen, P. B., Greengard, P., Rapp, P. R., Kordower, J. H., Hof, P. R., & Morrison, J. H. (2003). Estrogen increases the number of spinophilin-immunoreactive spines in the hippocampus of young and aged female rhesus monkeys. *The Journal of comparative neurology*, *465*(4), 540-550. https://doi.org/10.1002/cne.10837

Hauber, W. (1998). Involvement of basal ganglia transmitter systems in movement initiation. *Prog Neurobiol*, *56*(5), 507-540. https://doi.org/10.1016/s0301-0082(98)00041-0

Hawes, S. L., Evans, R. C., Unruh, B. A., Benkert, E. E., Gillani, F., Dumas, T. C., & Blackwell, K. T. (2015). Multimodal Plasticity in Dorsal Striatum While Learning a Lateralized Navigation Task. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *35*(29), 10535-10549. https://doi.org/10.1523/jneurosci.4415-14.2015

Heiman, M., Schaefer, A., Gong, S., Peterson, J. D., Day, M., Ramsey, K. E., Suárez-Fariñas, M., Schwarz, C., Stephan, D. A., Surmeier, D. J., Greengard, P., & Heintz, N. (2008). A translational profiling approach for the molecular characterization of CNS cell types. *Cell*, *135*(4), 738-748. https://doi.org/10.1016/j.cell.2008.10.028

Hernández-Echeagaray, E., Starling, A. J., Cepeda, C., & Levine, M. S. (2004). Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary? *Eur J Neurosci*, *19*(9), 2455-2463. https://doi.org/10.1111/j.0953-816X.2004.03344.x

Heroes, E., Lesage, B., Görnemann, J., Beullens, M., Van Meervelt, L., & Bollen, M. (2013). The PP1 binding code: a molecular-lego strategy that governs specificity. *Febs j*, *280*(2), 584-595. https://doi.org/10.1111/j.1742-4658.2012.08547.x

Hiday, A. C., Edler, M. C., Salek, A. B., Morris, C. W., Thang, M., Rentz, T. J., Rose, K. L., Jones, L. M., & Baucum, A. J., 2nd. (2017). Mechanisms and Consequences of Dopamine Depletion-Induced Attenuation of the Spinophilin/Neurofilament Medium Interaction. *Neural plasticity*, *2017*, 4153076-4153076. https://doi.org/10.1155/2017/4153076

Hikosaka, O., Kim, H. F., Yasuda, M., & Yamamoto, S. (2014). Basal ganglia circuits for reward value-guided behavior. *Annu Rev Neurosci*, *37*, 289-306. https://doi.org/10.1146/annurev-neuro-071013-013924

Holder, M. K., Veichweg, S. S., & Mong, J. A. (2015). Methamphetamine-enhanced female sexual motivation is dependent on dopamine and progesterone signaling in the medial amygdala. *Hormones and Behavior*, *67*, 1-11. https://doi.org/https://doi.org/10.1016/j.yhbeh.2014.10.004

Hooks, M. S., Jones, G. H., Neill, D. B., & Justice, J. B. (1992). Individual differences in amphetamine sensitization: Dose-dependent effects. *Pharmacology Biochemistry and Behavior*, *41*(1), 203-210. https://doi.org/https://doi.org/10.1016/0091-3057(92)90083-R

Hsieh-Wilson, L. C., Benfenati, F., Snyder, G. L., Allen, P. B., Nairn, A. C., & Greengard, P. (2003). Phosphorylation of Spinophilin Modulates Its Interaction

with Actin Filaments*. *Journal of Biological Chemistry*, *278*(2), 1186-1194. https://doi.org/https://doi.org/10.1074/jbc.M205754200

Hu, J., Vidovic, M., Chen, M. M., Lu, Q. Y., & Song, Z. M. (2008). Activation of alpha 2A adrenoceptors alters dendritic spine development and the expression of spinophilin in cultured cortical neurones. *Brain Res*, *1199*, 37-45. https://doi.org/10.1016/j.brainres.2007.12.073

Hu, X. T., & Wang, R. Y. (1988). Comparison of effects of D-1 and D-2 dopamine receptor agonists on neurons in the rat caudate putamen: an electrophysiological study. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *8*(11), 4340-4348. https://doi.org/10.1523/JNEUROSCI.08-11-04340.1988

Huang da, W., Sherman, B. T., & Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*, *4*(1), 44-57. https://doi.org/10.1038/nprot.2008.211

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2009). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic acids research*, *37*(1), 1-13. https://doi.org/10.1093/nar/gkn923

Ikemoto, S. (2002). Ventral striatal anatomy of locomotor activity induced by cocaine, D-amphetamine, dopamine and D1/D2 agonists. *Neuroscience*, *113*(4), 939-955. https://doi.org/10.1016/s0306-4522(02)00247-6

Ikemoto, S. (2003). Involvement of the olfactory tubercle in cocaine reward: intracranial self-administration studies. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *23*(28), 9305-9311. https://doi.org/10.1523/jneurosci.23-28-09305.2003

Ikemoto, S. (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev*, *56*(1), 27-78. https://doi.org/10.1016/j.brainresrev.2007.05.004

Ikemoto, S., Qin, M., & Liu, Z.-H. (2005). The Functional Divide for Primary Reinforcement of D-Amphetamine Lies between the Medial and Lateral Ventral Striatum: Is the Division of the Accumbens Core, Shell, and Olfactory Tubercle Valid? *The Journal of Neuroscience*, *25*(20), 5061. https://doi.org/10.1523/JNEUROSCI.0892-05.2005

Ikemoto, S., Yang, C., & Tan, A. (2015). Basal ganglia circuit loops, dopamine and motivation: A review and enquiry. *Behavioural Brain Research*, *290*, 17-31. https://doi.org/https://doi.org/10.1016/j.bbr.2015.04.018

Ingham, C. A., Hood, S. H., & Arbuthnott, G. W. (1989). Spine density on neostriatal neurones changes with 6-hydroxydopamine lesions and with age. *Brain research*, *503*(2), 334-338. https://doi.org/10.1016/0006-8993(89)91686-7

Ingham, C. A., Hood, S. H., van Maldegem, B., Weenink, A., & Arbuthnott, G. W. (1993). Morphological changes in the rat neostriatum after unilateral 6-hydroxydopamine injections into the nigrostriatal pathway. *Exp Brain Res*, *93*(1), 17-27. https://doi.org/10.1007/bf00227776

Jahanshahi, M., Jones, C. R. G., Dirnberger, G., & Frith, C. D. (2006). The Substantia Nigra Pars Compacta and Temporal Processing. *The Journal of Neuroscience*, *26*(47), 12266. https://doi.org/10.1523/JNEUROSCI.2540-06.2006

Jedynak, J., Hearing, M., Ingebretson, A., Ebner, S. R., Kelly, M., Fischer, R. A., Kourrich, S., & Thomas, M. J. (2016). Cocaine and Amphetamine Induce Overlapping but Distinct Patterns of AMPAR Plasticity in Nucleus Accumbens Medium Spiny Neurons. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *41*(2), 464-476. https://doi.org/10.1038/npp.2015.168

Jedynak, J. P., Uslaner, J. M., Esteban, J. A., & Robinson, T. E. (2007). Methamphetamine-induced structural plasticity in the dorsal striatum. *Eur J Neurosci*, *25*(3), 847-853. https://doi.org/10.1111/j.1460-9568.2007.05316.x

Jenkins, M. A., & Traynelis, S. F. (2012). PKC phosphorylates GluA1-Ser831 to enhance AMPA receptor conductance. *Channels (Austin, Tex.)*, *6*(1), 60-64. https://doi.org/10.4161/chan.18648

Kalivas, P. W. (2007). Cocaine and amphetamine-like psychostimulants: neurocircuitry and glutamate neuroplasticity. *Dialogues in clinical neuroscience*, *9*(4), 389-397. https://doi.org/10.31887/DCNS.2007.9.4/pkalivas

Kalivas, P. W., Lalumiere, R. T., Knackstedt, L., & Shen, H. (2009). Glutamate transmission in addiction. *Neuropharmacology*, *56 Suppl 1*(Suppl 1), 169-173. https://doi.org/10.1016/j.neuropharm.2008.07.011

Kalivas, P. W., & Stewart, J. (1991). Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Research Reviews*, *16*(3), 223-244. https://doi.org/https://doi.org/10.1016/0165-0173(91)90007-U

Kalivas, P. W., & Stewart, J. (1991). Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev*, *16*(3), 223-244. https://doi.org/10.1016/0165-0173(91)90007-u

Kalueff, A. V., Aldridge, J. W., LaPorte, J. L., Murphy, D. L., & Tuohimaa, P. (2007). Analyzing grooming microstructure in neurobehavioral experiments. *Nat Protoc*, *2*(10), 2538-2544. https://doi.org/10.1038/nprot.2007.367

Kalueff, A. V., Stewart, A. M., Song, C., Berridge, K. C., Graybiel, A. M., & Fentress, J. C. (2016). Neurobiology of rodent self-grooming and its value for translational neuroscience. *Nature reviews. Neuroscience*, *17*(1), 45-59. https://doi.org/10.1038/nrn.2015.8

Karakas, E., & Furukawa, H. (2014). Crystal structure of a heterotetrameric NMDA receptor ion channel. *Science (New York, N.Y.)*, *344*(6187), 992-997. https://doi.org/10.1126/science.1251915

Kass, R. S., Kurokawa, J., Marx, S. O., & Marks, A. R. (2003). Leucine/isoleucine zipper coordination of ion channel macromolecular signaling complexes in the heart. Roles in inherited arrhythmias. *Trends Cardiovasc Med*, *13*(2), 52-56. https://doi.org/10.1016/s1050-1738(02)00211-6

Kelker, M. S., Dancheck, B., Ju, T., Kessler, R. P., Hudak, J., Nairn, A. C., & Peti, W. (2007). Structural Basis for Spinophilin−Neurabin Receptor Interaction. *Biochemistry*, *46*(9), 2333-2344. https://doi.org/10.1021/bi602341c

Kerchner, G. A., & Nicoll, R. A. (2008). Silent synapses and the emergence of a postsynaptic mechanism for LTP. *Nature reviews. Neuroscience*, *9*(11), 813-825. https://doi.org/10.1038/nrn2501

Kimura, T., Allen, P. B., Nairn, A. C., & Caplan, M. J. (2007). Arrestins and spinophilin competitively regulate Na+,K+-ATPase trafficking through association with a large cytoplasmic loop of the Na+,K+-ATPase. *Molecular biology of the cell*, *18*(11), 4508-4518. https://doi.org/10.1091/mbc.e06-08-0711

King, L. J., Carl, J. L., & Lao, L. (1975). Cocaine and amphetamine modification of cerebral energy metabolism in vivo. *Psychopharmacologia*, *44*(1), 43-45. https://doi.org/10.1007/bf00421182

Kish, S. J., Shannak, K., & Hornykiewicz, O. (1988). Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. *N Engl J Med*, *318*(14), 876-880. https://doi.org/10.1056/nejm198804073181402

Kita, H., & Kita, T. (2011). Role of Striatum in the Pause and Burst Generation in the Globus Pallidus of 6-OHDA-Treated Rats [Original Research]. *Frontiers in Systems Neuroscience*, *5*. https://doi.org/10.3389/fnsys.2011.00042

Kita, T., Osten, P., & Kita, H. (2014). Rat subthalamic nucleus and zona incerta share extensively overlapped representations of cortical functional territories. *The Journal of comparative neurology*, *522*(18), 4043-4056. https://doi.org/10.1002/cne.23655

Klaus, A., Alves da Silva, J., & Costa, R. M. (2019). What, If, and When to Move: Basal Ganglia Circuits and Self-Paced Action Initiation. *Annu Rev Neurosci*, *42*, 459-483. https://doi.org/10.1146/annurev-neuro-072116-031033

Klongpanichapak, S., Phansuwan-Pujito, P., Ebadi, M., & Govitrapong, P. (2007). Melatonin protects SK-N-SH neuroblastoma cells from amphetamine-induced neurotoxicity. *J Pineal Res*, *43*(1), 65-73. https://doi.org/10.1111/j.1600-079X.2007.00444.x

Korchounov, A. M. (2008). Role of D1 and D2 receptors in the regulation of voluntary movements. *Bulletin of Experimental Biology and Medicine*, *146*(1), 14-17. https://doi.org/10.1007/s10517-008-0197-0

Kourrich, S., Klug, J. R., Mayford, M., & Thomas, M. J. (2012). AMPAR-independent effect of striatal αCaMKII promotes the sensitization of cocaine reward. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *32*(19), 6578-6586. https://doi.org/10.1523/JNEUROSCI.6391-11.2012

Kravitz, A. V., Freeze, B. S., Parker, P. R. L., Kay, K., Thwin, M. T., Deisseroth, K., & Kreitzer, A. C. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature*, *466*(7306), 622-626. https://doi.org/10.1038/nature09159

Kravitz, A. V., & Kreitzer, A. C. (2012). Striatal mechanisms underlying movement, reinforcement, and punishment. *Physiology (Bethesda, Md.)*, *27*(3), 167-177. https://doi.org/10.1152/physiol.00004.2012

Kreitzer, A. C., & Malenka, R. C. (2005). Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *25*(45), 10537-10545. https://doi.org/10.1523/JNEUROSCI.2959-05.2005

Kreitzer, A. C., & Malenka, R. C. (2008). Striatal plasticity and basal ganglia circuit function. *Neuron*, *60*(4), 543-554. https://doi.org/10.1016/j.neuron.2008.11.005

Lai, S., Zhong, S., Liao, X., Wang, Y., Huang, J., Zhang, S., Sun, Y., Zhao, H., & Jia, Y. (2018). Biochemical abnormalities in basal ganglia and executive dysfunction in acute- and euthymic-episode patients with bipolar disorder: A proton magnetic resonance spectroscopy study. *J Affect Disord*, *225*, 108-116. https://doi.org/10.1016/j.jad.2017.07.036

Lanciego, J. L., Luquin, N., & Obeso, J. A. (2012). Functional neuroanatomy of the basal ganglia. *Cold Spring Harbor perspectives in medicine*, *2*(12), a009621-a009621. https://doi.org/10.1101/cshperspect.a009621

Lee, C., Witkovsky, P., & Rice, M. (2011). Regulation of Substantia Nigra Pars Reticulata GABAergic Neuron Activity by H2O2 via Flufenamic Acid-Sensitive Channels and KATP Channels [Original Research]. *Frontiers in Systems Neuroscience*, *5*(14). https://doi.org/10.3389/fnsys.2011.00014

Lee, H. J., Weitz, A. J., Bernal-Casas, D., Duffy, B. A., Choy, M., Kravitz, A. V., Kreitzer, A. C., & Lee, J. H. (2016). Activation of Direct and Indirect Pathway Medium Spiny Neurons Drives Distinct Brain-wide Responses. *Neuron*, *91*(2), 412-424. https://doi.org/10.1016/j.neuron.2016.06.010

Lee, K.-W., Kim, Y., Kim, A. M., Helmin, K., Nairn, A. C., & Greengard, P. (2006). Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(9), 3399-3404. https://doi.org/10.1073/pnas.0511244103

Lee, S. J., Romeo, R. D., Svenningsson, P., Campomanes, C. R., Allen, P. B., Greengard, P., & McEwen, B. S. (2004). Estradiol affects spinophilin protein differently in gonadectomized males and females. *Neuroscience*, *127*(4), 983-988. https://doi.org/10.1016/j.neuroscience.2004.05.049

Leisman, G., Braun-Benjamin, O., & Melillo, R. (2014). Cognitive-motor interactions of the basal ganglia in development [Review]. *Frontiers in Systems Neuroscience*, *8*(16). https://doi.org/10.3389/fnsys.2014.00016

Lewis, R. G., Serra, M., Radl, D., Gori, M., Tran, C., Michalak, S. E., Vanderwal, C. D., & Borrelli, E. (2020). Dopaminergic Control of Striatal Cholinergic Interneurons Underlies Cocaine-Induced Psychostimulation. *Cell reports*, *31*(3), 107527. https://doi.org/10.1016/j.celrep.2020.107527

Li, Y., Kolb, B., & Robinson, T. E. (2003). The location of persistent amphetamine-induced changes in the density of dendritic spines on medium spiny neurons in the nucleus accumbens and caudate-putamen. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *28*(6), 1082-1085. https://doi.org/10.1038/sj.npp.1300115

Liang, L., DeLong, M. R., & Papa, S. M. (2008). Inversion of dopamine responses in striatal medium spiny neurons and involuntary movements. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *28*(30), 7537-7547. https://doi.org/10.1523/JNEUROSCI.1176-08.2008

Lindskog, M., Kim, M., Wikström, M. A., Blackwell, K. T., & Kotaleski, J. H. (2006). Transient calcium and dopamine increase PKA activity and DARPP-32 phosphorylation. *PLoS computational biology*, *2*(9), e119-e119. https://doi.org/10.1371/journal.pcbi.0020119

Lobo, M. K., & Nestler, E. (2011). The Striatal Balancing Act in Drug Addiction: Distinct Roles of Direct and Indirect Pathway Medium Spiny Neurons [Review]. *Frontiers in Neuroanatomy*, *5*. https://doi.org/10.3389/fnana.2011.00041

Loweth, J. A., Singer, B. F., Baker, L. K., Wilke, G., Inamine, H., Bubula, N., Alexander, J. K., Carlezon, W. A., Neve, R. L., & Vezina, P. (2010). Transient Overexpression of α-Ca²⁺/Calmodulin-Dependent Protein Kinase II in the Nucleus Accumbens Shell Enhances Behavioral Responding to Amphetamine. *The Journal of Neuroscience*, *30*(3), 939. https://doi.org/10.1523/JNEUROSCI.4383-09.2010

Lu, R., Chen, Y., Cottingham, C., Peng, N., Jiao, K., Limbird, L. E., Wyss, J. M., & Wang, Q. (2010). Enhanced hypotensive, bradycardic, and hypnotic responses to alpha2-adrenergic agonists in spinophilin-null mice are accompanied by increased G protein coupling to the alpha2A-adrenergic receptor. *Molecular pharmacology*, *78*(2), 279-286. https://doi.org/10.1124/mol.110.065300

Luft, A. R., & Buitrago, M. M. (2005). Stages of motor skill learning. *Mol Neurobiol*, *32*(3), 205-216. https://doi.org/10.1385/mn:32:3:205

MacMillan, L. B., Bass, M. A., Cheng, N., Howard, E. F., Tamura, M., Strack, S., Wadzinski, B. E., & Colbran, R. J. (1999). Brain actin-associated protein phosphatase 1 holoenzymes containing spinophilin, neurabin, and selected catalytic subunit isoforms. *J Biol Chem*, *274*(50), 35845-35854. https://doi.org/10.1074/jbc.274.50.35845

Malenka, R. C., & Bear, M. F. (2004). LTP and LTD: an embarrassment of riches. *Neuron*, *44*(1), 5-21. https://doi.org/10.1016/j.neuron.2004.09.012

Mallet, N., Micklem, B. R., Henny, P., Brown, M. T., Williams, C., Bolam, J. P., Nakamura, K. C., & Magill, P. J. (2012). Dichotomous organization of the external globus pallidus. *Neuron*, *74*(6), 1075-1086. https://doi.org/10.1016/j.neuron.2012.04.027

Mallet, N., Pogosyan, A., Márton, L. F., Bolam, J. P., Brown, P., & Magill, P. J. (2008). Parkinsonian Beta Oscillations in the External Globus Pallidus and Their Relationship with Subthalamic Nucleus Activity. *The Journal of Neuroscience*, *28*(52), 14245. https://doi.org/10.1523/JNEUROSCI.4199-08.2008

Mayer, B. J., & Eck, M. J. (1995). SH3 Domains: Minding your p's and q's. *Current Biology*, *5*(4), 364-367. https://doi.org/10.1016/S0960-9822(95)00073-X

McGregor, M. M., & Nelson, A. B. (2019). Circuit Mechanisms of Parkinson's Disease. *Neuron*, *101*(6), 1042-1056. https://doi.org/https://doi.org/10.1016/j.neuron.2019.03.004

Meitzen, J., Meisel, R. L., & Mermelstein, P. G. (2018). Sex Differences and the Effects of Estradiol on Striatal Function. *Current opinion in behavioral sciences*, *23*, 42-48. https://doi.org/10.1016/j.cobeha.2018.03.007

Mellacheruvu, D., Wright, Z., Couzens, A. L., Lambert, J.-P., St-Denis, N. A., Li, T., Miteva, Y. V., Hauri, S., Sardiu, M. E., Low, T. Y., Halim, V. A., Bagshaw, R. D., Hubner, N. C., Al-Hakim, A., Bouchard, A., Faubert, D., Fermin, D., Dunham, W. H., Goudreault, M., . . . Nesvizhskii, A. I. (2013). The CRAPome: a contaminant repository for affinity purification-mass spectrometry data. *Nature methods*, *10*(8), 730-736. https://doi.org/10.1038/nmeth.2557

Mink, J. W. (1996). THE BASAL GANGLIA: FOCUSED SELECTION AND INHIBITION OF COMPETING MOTOR PROGRAMS. *Progress in Neurobiology*, *50*(4), 381-425. https://doi.org/https://doi.org/10.1016/S0301-0082(96)00042-1

Molina-Luna, K., Pekanovic, A., Röhrich, S., Hertler, B., Schubring-Giese, M., Rioult-Pedotti, M.-S., & Luft, A. R. (2009). Dopamine in motor cortex is necessary for skill learning and synaptic plasticity. *PloS one*, *4*(9), e7082-e7082. https://doi.org/10.1371/journal.pone.0007082

Morishita, W., Connor, J. H., Xia, H., Quinlan, E. M., Shenolikar, S., & Malenka, R. C. (2001). Regulation of synaptic strength by protein phosphatase 1. *Neuron*, *32*(6), 1133-1148. https://doi.org/10.1016/s0896-6273(01)00554-2

Morris, C. W., Watkins, D. S., Salek, A. B., Edler, M. C., & Baucum, A. J., 2nd. (2018). The association of spinophilin with disks large-associated protein 3 (SAPAP3) is regulated by metabotropic glutamate receptor (mGluR) 5. *Molecular and cellular neurosciences*, *90*, 60-69. https://doi.org/10.1016/j.mcn.2018.06.001

Mosley, A. L., Sardiu, M. E., Pattenden, S. G., Workman, J. L., Florens, L., & Washburn, M. P. (2011). Highly reproducible label free quantitative proteomic analysis of RNA polymerase complexes. *Molecular & cellular proteomics : MCP*, *10*(2), M110.000687-M000110.000687. https://doi.org/10.1074/mcp.M110.000687

Muhammad, K., Reddy-Alla, S., Driller, J. H., Schreiner, D., Rey, U., Böhme, M. A., Hollmann, C., Ramesh, N., Depner, H., Lützkendorf, J., Matkovic, T., Götz, T., Bergeron, D. D., Schmoranzer, J., Goettfert, F., Holt, M., Wahl, M. C., Hell, S. W., Scheiffele, P., . . . Sigrist, S. J. (2015). Presynaptic spinophilin tunes neurexin signalling to control active zone architecture and function. *Nature communications*, *6*, 8362-8362. https://doi.org/10.1038/ncomms9362

Muly, E. C., Allen, P., Mazloom, M., Aranbayeva, Z., Greenfield, A. T., & Greengard, P. (2004). Subcellular Distribution of Neurabin Immunolabeling in Primate Prefrontal Cortex: Comparison with Spinophilin. *Cerebral Cortex*, *14*(12), 1398-1407. https://doi.org/10.1093/cercor/bhh101

Muly, E. C., Smith, Y., Allen, P., & Greengard, P. (2004). Subcellular distribution of spinophilin immunolabeling in primate prefrontal cortex: localization to and within dendritic spines. *The Journal of comparative neurology*, *469*(2), 185-197. https://doi.org/10.1002/cne.11001

Munton, R. P., Vizi, S., & Mansuy, I. M. (2004). The role of protein phosphatase-1 in the modulation of synaptic and structural plasticity. *FEBS Letters*, *567*(1), 121-128. https://doi.org/10.1016/j.febslet.2004.03.121

Muralidharan, A., Jensen, A. L., Connolly, A., Hendrix, C. M., Johnson, M. D., Baker, K. B., & Vitek, J. L. (2016). Physiological changes in the pallidum in a progressive model of Parkinson's disease: Are oscillations enough? *Exp Neurol*, *279*, 187-196. https://doi.org/10.1016/j.expneurol.2016.03.002

Nambu, A. (2007). Globus pallidus internal segment. In J. M. Tepper, E. D. Abercrombie, & J. P. Bolam (Eds.), *Progress in brain research* (Vol. 160, pp. 135-150). Elsevier. https://doi.org/https://doi.org/10.1016/S0079-6123(06)60008-3

Natarajan, R., & Yamamoto, B. K. (2011). The Basal Ganglia as a Substrate for the Multiple Actions of Amphetamines. *Basal ganglia*, *1*(2), 49-57. https://doi.org/10.1016/j.baga.2011.05.003

Neumann, W.-J., Schroll, H., de Almeida Marcelino, A. L., Horn, A., Ewert, S., Irmen, F., Krause, P., Schneider, G.-H., Hamker, F., & Kühn, A. A. (2018). Functional segregation of basal ganglia pathways in Parkinson's disease. *Brain*, *141*(9), 2655-2669. https://doi.org/10.1093/brain/awy206

Nieto Mendoza, E., & Hernández Echeagaray, E. (2015). Dopaminergic Modulation of Striatal Inhibitory Transmission and Long-Term Plasticity. *Neural plasticity*, *2015*, 789502-789502. https://doi.org/10.1155/2015/789502

Nishi, A., Matamales, M., Musante, V., Valjent, E., Kuroiwa, M., Kitahara, Y., Rebholz, H., Greengard, P., Girault, J.-A., & Nairn, A. C. (2017). Glutamate Counteracts Dopamine/PKA Signaling via Dephosphorylation of DARPP-32 Ser-97 and Alteration of Its Cytonuclear Distribution. *The Journal of biological chemistry*, *292*(4), 1462-1476. https://doi.org/10.1074/jbc.M116.752402

Ogura, T., Ogata, M., Akita, H., Jitsuki, S., Akiba, L., Noda, K., Hoka, S., & Saji, M. (2005). Impaired acquisition of skilled behavior in rotarod task by moderate depletion of striatal dopamine in a pre-symptomatic stage model of Parkinson's disease. *Neurosci Res*, *51*(3), 299-308. https://doi.org/10.1016/j.neures.2004.12.006

Ohtsuka, N., Tansky Morris, F., Kuang, H., Kourrich, S., Thomas Mark, J., Rubenstein John, L. R., Ekker, M., Leeman Susan, E., & Tsien Joe, Z. (2008). Functional disturbances in the striatum by region-specific ablation of NMDA receptors. *Proceedings of the National Academy of Sciences*, *105*(35), 12961-12966. https://doi.org/10.1073/pnas.0806180105

Oliver, C. J., Terry-Lorenzo, R. T., Elliott, E., Bloomer, W. A. C., Li, S., Brautigan, D. L., Colbran, R. J., & Shenolikar, S. (2002). Targeting protein phosphatase 1 (PP1) to the actin cytoskeleton: the neurabin I/PP1 complex regulates cell morphology. *Molecular and cellular biology*, *22*(13), 4690-4701. https://doi.org/10.1128/MCB.22.13.4690-4701.2002

Ouimet, C. C., da Cruz e Silva, E. F., & Greengard, P. (1995). The alpha and gamma 1 isoforms of protein phosphatase 1 are highly and specifically concentrated in dendritic spines. *Proceedings of the National Academy of Sciences*, *92*(8), 3396-3400. https://doi.org/10.1073/pnas.92.8.3396

Paoletti, P., Bellone, C., & Zhou, Q. (2013). NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nature Reviews Neuroscience*, *14*(6), 383-400. https://doi.org/10.1038/nrn3504

Perrin, E., & Venance, L. (2019). Bridging the gap between striatal plasticity and learning. *Current opinion in neurobiology*, *54*, 104-112. https://doi.org/https://doi.org/10.1016/j.conb.2018.09.007

Peti, W., Nairn, A. C., & Page, R. (2013). Structural basis for protein phosphatase 1 regulation and specificity. *Febs j*, *280*(2), 596-611. https://doi.org/10.1111/j.1742-4658.2012.08509.x

Phillips, K. A., Epstein, D. H., & Preston, K. L. (2014). Psychostimulant addiction treatment. *Neuropharmacology*, *87*, 150-160. https://doi.org/10.1016/j.neuropharm.2014.04.002

Pizzo, A. B., Karam, C. S., Zhang, Y., Ma, C. L., McCabe, B. D., & Javitch, J. A. (2014). Amphetamine-induced behavior requires CaMKII-dependent dopamine transporter phosphorylation. *Molecular Psychiatry*, *19*(3), 279-281. https://doi.org/10.1038/mp.2013.29

Porter, D. E., Cohen, B. B., Wallace, M. R., Smyth, E., Chetty, U., Dixon, J. M., Steel, C. M., & Carter, D. C. (1994). Breast cancer incidence, penetrance and survival in probable carriers of BRCA1 gene mutation in families linked to BRCA1 on chromosome 17q12–21 [https://doi.org/10.1002/bjs.1800811038]. *BJS (British Journal of Surgery)*, *81*(10), 1512-1515. https://doi.org/https://doi.org/10.1002/bjs.1800811038

Price, C. J., Kim, P., & Raymond, L. A. (1999). D1 dopamine receptor-induced cyclic AMP-dependent protein kinase phosphorylation and potentiation of striatal glutamate receptors. *J Neurochem*, *73*(6), 2441-2446. https://doi.org/10.1046/j.1471-4159.1999.0732441.x

Qian, Y., Forssberg, H., & Diaz Heijtz, R. (2015). Motor Skill Learning Is Associated with Phase-Dependent Modifications in the Striatal cAMP/PKA/DARPP-32 Signaling Pathway in Rodents. *PloS one*, *10*(10), e0140974-e0140974. https://doi.org/10.1371/journal.pone.0140974

Ragusa, M. J., Allaire, M., Nairn, A. C., Page, R., & Peti, W. (2011). Flexibility in the PP1:spinophilin holoenzyme. *FEBS Lett*, *585*(1), 36-40. https://doi.org/10.1016/j.febslet.2010.11.022

Ragusa, M. J., Dancheck, B., Critton, D. A., Nairn, A. C., Page, R., & Peti, W. (2010). Spinophilin directs protein phosphatase 1 specificity by blocking substrate binding sites. *Nature structural & molecular biology*, *17*(4), 459-464. https://doi.org/10.1038/nsmb.1786

Rajadhyaksha, A., Leveque, J., Macías, W., Barczak, A., & Konradi, C. (1998). Molecular components of striatal plasticity: the various routes of cyclic AMP pathways. *Developmental neuroscience*, *20*(2-3), 204-215. https://doi.org/10.1159/000017314

Ravenscroft, P., & Brotchie, J. (2000). NMDA receptors in the basal ganglia. *Journal of anatomy*, *196 (Pt 4)*(Pt 4), 577-585. https://doi.org/10.1046/j.1469-7580.2000.19640577.x

Rebec, G. V. (2006). Behavioral electrophysiology of psychostimulants. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *31*(11), 2341-2348. https://doi.org/10.1038/sj.npp.1301160

Rebelo, S., Santos, M., Martins, F., da Cruz e Silva, E. F., & da Cruz e Silva, O. A. B. (2015). Protein phosphatase 1 is a key player in nuclear events. *Cellular Signalling*, *27*(12), 2589-2598. https://doi.org/https://doi.org/10.1016/j.cellsig.2015.08.007

Redgrave, P., Rodriguez, M., Smith, Y., Rodriguez-Oroz, M. C., Lehericy, S., Bergman, H., Agid, Y., DeLong, M. R., & Obeso, J. A. (2010). Goal-directed and habitual control in the basal ganglia: implications for Parkinson's disease. *Nature Reviews Neuroscience*, *11*(11), 760-772. https://doi.org/10.1038/nrn2915

Richman, J. G., Brady, A. E., Wang, Q., Hensel, J. L., Colbran, R. J., & Limbird, L. E. (2001). Agonist-regulated Interaction between alpha2-adrenergic receptors and

spinophilin. *J Biol Chem*, *276*(18), 15003-15008. https://doi.org/10.1074/jbc.M011679200

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

Rioult-Pedotti, M.-S., Pekanovic, A., Atiemo, C. O., Marshall, J., & Luft, A. R. (2015). Dopamine Promotes Motor Cortex Plasticity and Motor Skill Learning via PLC Activation. *PloS one*, *10*(5), e0124986-e0124986. https://doi.org/10.1371/journal.pone.0124986

Robinson, T. E., & Becker, J. B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. *Brain Research Reviews*, *11*(2), 157-198. https://doi.org/https://doi.org/10.1016/0165-0173(86)90002-0

Rothwell, P. E., Hayton, S. J., Sun, G. L., Fuccillo, M. V., Lim, B. K., & Malenka, R. C. (2015). Input- and Output-Specific Regulation of Serial Order Performance by Corticostriatal Circuits. *Neuron*, *88*(2), 345-356. https://doi.org/10.1016/j.neuron.2015.09.035

Ruiz de Azua, I., Nakajima, K.-i., Rossi, M., Cui, Y., Jou, W., Gavrilova, O., & Wess, J. (2012). Spinophilin as a novel regulator of M3 muscarinic receptor-mediated insulin release in vitro and in vivo. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, *26*(10), 4275-4286. https://doi.org/10.1096/fj.12-204644

Ryan, M. B., Bair-Marshall, C., & Nelson, A. B. (2018). Aberrant Striatal Activity in Parkinsonism and Levodopa-Induced Dyskinesia. *Cell reports*, *23*(12), 3438-3446.e3435. https://doi.org/10.1016/j.celrep.2018.05.059

Sagot, B., Li, L., & Zhou, F.-M. (2018). Hyperactive Response of Direct Pathway Striatal Projection Neurons to L-dopa and D1 Agonism in Freely Moving Parkinsonian Mice. *Frontiers in neural circuits*, *12*, 57-57. https://doi.org/10.3389/fncir.2018.00057

Sakayori, N., Kato, S., Sugawara, M., Setogawa, S., Fukushima, H., Ishikawa, R., Kida, S., & Kobayashi, K. (2019). Motor skills mediated through cerebellothalamic tracts projecting to the central lateral nucleus. *Molecular Brain*, *12*(1), 13. https://doi.org/10.1186/s13041-019-0431-x

Salek, A. B., Edler, M. C., McBride, J. P., & Baucum, A. J., 2nd. (2019). Spinophilin regulates phosphorylation and interactions of the GluN2B subunit of the N-methyl-d-aspartate receptor. *Journal of neurochemistry*, *151*(2), 185-203. https://doi.org/10.1111/jnc.14831

Salery, M., Trifilieff, P., Caboche, J., & Vanhoutte, P. (2020). From Signaling Molecules to Circuits and Behaviors: Cell-Type-Specific Adaptations to Psychostimulant Exposure in the Striatum. *Biological psychiatry*, *87*(11), 944-953. https://doi.org/10.1016/j.biopsych.2019.11.001

Sanchez-Ramos, J. (2015). Chapter Seven - Neurologic Complications of Psychomotor Stimulant Abuse. In P. Taba, A. Lees, & K. Sikk (Eds.), *International Review of Neurobiology* (Vol. 120, pp. 131-160). Academic Press. https://doi.org/https://doi.org/10.1016/bs.irn.2015.02.003

Sarrouilhe, D., di Tommaso, A., Métayé, T., & Ladeveze, V. (2006). Spinophilin: from partners to functions. *Biochimie*, *88*(9), 1099-1113. https://doi.org/https://doi.org/10.1016/j.biochi.2006.04.010

Satoh, A., Nakanishi, H., Obaishi, H., Wada, M., Takahashi, K., Satoh, K., Hirao, K., Nishioka, H., Hata, Y., Mizoguchi, A., & Takai, Y. (1998). Neurabin-II/Spinophilin: AN ACTIN FILAMENT-BINDING PROTEIN WITH ONE PDZ DOMAIN LOCALIZED AT CADHERIN-BASED CELL-CELL ADHESION SITES*. *Journal of Biological Chemistry*, *273*(6), 3470-3475. https://doi.org/https://doi.org/10.1074/jbc.273.6.3470

Saxena, S., Bota, R. G., & Brody, A. L. (2001). Brain-behavior relationships in obsessive-compulsive disorder. *Semin Clin Neuropsychiatry*, *6*(2), 82-101. https://doi.org/10.1053/scnp.2001.21833

Schilman, E. A., Klavir, O., Winter, C., Sohr, R., & Joel, D. (2010). The role of the striatum in compulsive behavior in intact and orbitofrontal-cortex-lesioned rats: possible involvement of the serotonergic system. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *35*(4), 1026-1039. https://doi.org/10.1038/npp.2009.208

Schmidt, L. S., Miller, A. D., Lester, D. B., Bay-Richter, C., Schülein, C., Frikke-Schmidt, H., Wess, J., Blaha, C. D., Woldbye, D. P. D., Fink-Jensen, A., & Wortwein, G. (2010). Increased amphetamine-induced locomotor activity, sensitization, and accumbal dopamine release in M5 muscarinic receptor knockout mice. *Psychopharmacology*, *207*(4), 547-558. https://doi.org/10.1007/s00213-009-1685-2

Schneck, N., & Vezina, P. (2012). Enhanced dorsolateral striatal activity in drug use: the role of outcome in stimulus-response associations. *Behavioural Brain Research*, *235*(2), 136-142. https://doi.org/10.1016/j.bbr.2012.07.042

Scholz, J., Niibori, Y., P, W. F., & J, P. L. (2015). Rotarod training in mice is associated with changes in brain structure observable with multimodal MRI. *Neuroimage*, *107*, 182-189. https://doi.org/10.1016/j.neuroimage.2014.12.003

Schultz, W. (2016). Reward functions of the basal ganglia. *J Neural Transm (Vienna)*, *123*(7), 679-693. https://doi.org/10.1007/s00702-016-1510-0

Schwartz, A. B. (2016). Movement: How the Brain Communicates with the World. *Cell*, *164*(6), 1122-1135. https://doi.org/10.1016/j.cell.2016.02.038

Scicutella, A. (2000). Late-life obsessive-compulsive disorder and Huntington's disease. *J Neuropsychiatry Clin Neurosci*, *12*(2), 288-289. https://doi.org/10.1176/jnp.12.2.288

Shan, Q., Christie, M. J., & Balleine, B. W. (2015). Plasticity in striatopallidal projection neurons mediates the acquisition of habitual actions. *Eur J Neurosci*, *42*(4), 2097-2104. https://doi.org/10.1111/ejn.12971

Shan, Q., Ge, M., Christie, M. J., & Balleine, B. W. (2014). The acquisition of goal-directed actions generates opposing plasticity in direct and indirect pathways in dorsomedial striatum. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *34*(28), 9196-9201. https://doi.org/10.1523/jneurosci.0313-14.2014

Shen, W., Flajolet, M., Greengard, P., & Surmeier, D. J. (2008). Dichotomous dopaminergic control of striatal synaptic plasticity. *Science (New York, N.Y.)*, *321*(5890), 848-851. https://doi.org/10.1126/science.1160575

Shen, W., Tian, X., Day, M., Ulrich, S., Tkatch, T., Nathanson, N. M., & Surmeier, D. J. (2007). Cholinergic modulation of Kir2 channels selectively elevates dendritic excitability in striatopallidal neurons. *Nat Neurosci*, *10*(11), 1458-1466. https://doi.org/10.1038/nn1972

Shin, E., Kashiwagi, Y., Kuriu, T., Iwasaki, H., Tanaka, T., Koizumi, H., Gleeson, J. G., & Okabe, S. (2013). Doublecortin-like kinase enhances dendritic remodelling and negatively regulates synapse maturation. *Nature communications*, *4*, 1440-1440. https://doi.org/10.1038/ncomms2443

Shmelkov, S. V., Hormigo, A., Jing, D., Proenca, C. C., Bath, K. G., Milde, T., Shmelkov, E., Kushner, J. S., Baljevic, M., Dincheva, I., Murphy, A. J., Valenzuela, D. M., Gale, N. W., Yancopoulos, G. D., Ninan, I., Lee, F. S., & Rafii, S. (2010). Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsive-like behaviors in mice. *Nature medicine*, *16*(5), 598-602. https://doi.org/10.1038/nm.2125

Singer, B. F., Tanabe, L. M., Gorny, G., Jake-Matthews, C., Li, Y., Kolb, B., & Vezina, P. (2009). Amphetamine-induced changes in dendritic morphology in rat forebrain correspond to associative drug conditioning rather than nonassociative drug sensitization. *Biological psychiatry*, *65*(10), 835-840. https://doi.org/10.1016/j.biopsych.2008.12.020

Siviy, S. M., McDowell, L. S., Eck, S. R., Turano, A., Akopian, G., & Walsh, J. P. (2015). Effects of amphetamine on striatal dopamine release, open-field activity, and play in Fischer 344 and Sprague-Dawley rats. *Behav Pharmacol*, *26*(8 Spec No), 720-732. https://doi.org/10.1097/fbp.0000000000000191

Smith, F. D., Oxford, G. S., & Milgram, S. L. (1999). Association of the D2 dopamine receptor third cytoplasmic loop with spinophilin, a protein phosphatase-1-interacting protein. *J Biol Chem*, *274*(28), 19894-19900. https://doi.org/10.1074/jbc.274.28.19894

Smith-Kinnaman, W. R., Berna, M. J., Hunter, G. O., True, J. D., Hsu, P., Cabello, G. I., Fox, M. J., Varani, G., & Mosley, A. L. (2014). The interactome of the atypical phosphatase Rtr1 in Saccharomyces cerevisiae. *Molecular bioSystems*, *10*(7), 1730-1741. https://doi.org/10.1039/c4mb00109e

Snyder, G. L., Allen, P. B., Fienberg, A. A., Valle, C. G., Huganir, R. L., Nairn, A. C., & Greengard, P. (2000). Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *20*(12), 4480-4488. https://doi.org/10.1523/JNEUROSCI.20-12-04480.2000

Soares, J., Kliem, M. A., Betarbet, R., Greenamyre, J. T., Yamamoto, B., & Wichmann, T. (2004). Role of external pallidal segment in primate parkinsonism: comparison of the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism and lesions of the external pallidal segment. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *24*(29), 6417-6426. https://doi.org/10.1523/JNEUROSCI.0836-04.2004

Sonne J, R. V., Beato M.R. (2020). *Neuroanatomy, Substantia Nigra*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK536995/

Stalnaker, T. A., Calhoon, G. G., Ogawa, M., Roesch, M. R., & Schoenbaum, G. (2012). Reward Prediction Error Signaling in Posterior Dorsomedial Striatum Is Action Specific. *The Journal of Neuroscience*, *32*(30), 10296. https://doi.org/10.1523/JNEUROSCI.0832-12.2012

Starr, P. A., Rau, G. M., Davis, V., Marks, W. J., Jr., Ostrem, J. L., Simmons, D., Lindsey, N., & Turner, R. S. (2005). Spontaneous pallidal neuronal activity in human dystonia: comparison with Parkinson's disease and normal macaque. *J Neurophysiol*, *93*(6), 3165-3176. https://doi.org/10.1152/jn.00971.2004

Steketee, J. D., & Kalivas, P. W. (2011). Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. *Pharmacological reviews*, *63*(2), 348-365. https://doi.org/10.1124/pr.109.001933

Strack, S., Kini, S., Ebner, F. F., Wadzinski, B. E., & Colbran, R. J. (1999). Differential cellular and subcellular localization of protein phosphatase 1 isoforms in brain. *The Journal of comparative neurology*, *413*(3), 373-384.

Straiko, M. M. W., Coolen, L. M., Zemlan, F. P., & Gudelsky, G. A. (2007). The effect of amphetamine analogs on cleaved microtubule-associated protein-tau formation in the rat brain. *Neuroscience*, *144*(1), 223-231. https://doi.org/10.1016/j.neuroscience.2006.08.073

Studelska, D. R., & Beatty, W. W. (1978). Open-field and avoidance behavior after neostriatal lesions in male and female rats. *J Comp Physiol Psychol*, *92*(2), 297-311. https://doi.org/10.1037/h0077463

Subramanian, K., Brandenburg, C., Orsati, F., Soghomonian, J. J., Hussman, J. P., & Blatt, G. J. (2017). Basal ganglia and autism - a translational perspective. *Autism Res*, *10*(11), 1751-1775. https://doi.org/10.1002/aur.1837

Surmeier, D. J., Ding, J., Day, M., Wang, Z., & Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci*, *30*(5), 228-235. https://doi.org/10.1016/j.tins.2007.03.008

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

Svenningsson, P., Nairn, A. C., & Greengard, P. (2005). DARPP-32 mediates the actions of multiple drugs of abuse. *The AAPS Journal*, *7*(2), E353-E360. https://doi.org/10.1208/aapsj070235

Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K. P., Kuhn, M., Bork, P., Jensen, L. J., & von Mering, C. (2015). STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic acids research*, *43*(Database issue), D447-D452. https://doi.org/10.1093/nar/gku1003

Szklarczyk, D., Morris, J. H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., Santos, A., Doncheva, N. T., Roth, A., Bork, P., Jensen, L. J., & von Mering, C. (2017). The STRING database in 2017: quality-controlled protein-protein association

networks, made broadly accessible. *Nucleic acids research*, *45*(D1), D362-D368. https://doi.org/10.1093/nar/gkw937

Tang, K.-C., Low Malcolm, J., Grandy David, K., & Lovinger David, M. (2001). Dopamine-dependent synaptic plasticity in striatum during in vivo development. *Proceedings of the National Academy of Sciences*, *98*(3), 1255-1260. https://doi.org/10.1073/pnas.98.3.1255

Taylor, J. L., Rajbhandari, A. K., Berridge, K. C., & Aldridge, J. W. (2010). Dopamine receptor modulation of repetitive grooming actions in the rat: potential relevance for Tourette syndrome. *Brain research*, *1322*, 92-101. https://doi.org/10.1016/j.brainres.2010.01.052

Tepper, J. M., & Koós, T. (2016). Chapter 8 - GABAergic Interneurons of the Striatum. In H. Steiner & K. Y. Tseng (Eds.), *Handbook of Behavioral Neuroscience* (Vol. 24, pp. 157-178). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-802206-1.00008-8

Terry-Lorenzo, R. T., Carmody, L. C., Voltz, J. W., Connor, J. H., Li, S., Smith, F. D., Milgram, S. L., Colbran, R. J., & Shenolikar, S. (2002). The Neuronal Actin-binding Proteins, Neurabin I and Neurabin II, Recruit Specific Isoforms of Protein Phosphatase-1 Catalytic Subunits*. *Journal of Biological Chemistry*, *277*(31), 27716-27724. https://doi.org/https://doi.org/10.1074/jbc.M203365200

Toda, S., Shen, H.-W., Peters, J., Cagle, S., & Kalivas, P. W. (2006). Cocaine increases actin cycling: effects in the reinstatement model of drug seeking. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *26*(5), 1579-1587. https://doi.org/10.1523/JNEUROSCI.4132-05.2006

Ukken, F. P., Bruckner, J. J., Weir, K. L., Hope, S. J., Sison, S. L., Birschbach, R. M., Hicks, L., Taylor, K. L., Dent, E. W., Gonsalvez, G. B., & O'Connor-Giles, K. M. (2016). BAR-SH3 sorting nexins are conserved interacting proteins of Nervous wreck that organize synapses and promote neurotransmission. *Journal of cell science*, *129*(1), 166-177. https://doi.org/10.1242/jcs.178699

Ungless, M. A., Whistler, J. L., Malenka, R. C., & Bonci, A. (2001). Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature*, *411*(6837), 583-587. https://doi.org/10.1038/35079077

Valjent, E., Corvol, J.-C., Trzaskos, J. M., Girault, J.-A., & Hervé, D. (2006). Role of the ERK pathway in psychostimulant-induced locomotor sensitization. *BMC Neuroscience*, *7*(1), 20. https://doi.org/10.1186/1471-2202-7-20

Valvassori, S. S., Calixto, K. V., Budni, J., Resende, W. R., Varela, R. B., de Freitas, K. V., Gonçalves, C. L., Streck, E. L., & Quevedo, J. (2013). Sodium butyrate reverses the inhibition of Krebs cycle enzymes induced by amphetamine in the rat brain. *J Neural Transm (Vienna)*, *120*(12), 1737-1742. https://doi.org/10.1007/s00702-013-1056-3

Verdugo-Sivianes, E. M., & Carnero, A. (2022). SPINOPHILIN: A multiplayer tumor suppressor. *Genes & Diseases*. https://doi.org/https://doi.org/10.1016/j.gendis.2021.12.021

Vezina, P. (2007). Sensitization, drug addiction and psychopathology in animals and humans. *Progress in neuro-psychopharmacology & biological psychiatry*, *31*(8), 1553-1555. https://doi.org/10.1016/j.pnpbp.2007.08.030

Volkow, N. D., Wang, G.-J., Telang, F., Fowler, J. S., Logan, J., Childress, A.-R., Jayne, M., Ma, Y., & Wong, C. (2006). Cocaine Cues and Dopamine in Dorsal Striatum: Mechanism of Craving in Cocaine Addiction. *The Journal of Neuroscience*, *26*(24), 6583. https://doi.org/10.1523/JNEUROSCI.1544-06.2006

Volkow, N. D., Wang, G. J., Fowler, J. S., Logan, J., Gatley, S. J., Wong, C., Hitzemann, R., & Pappas, N. R. (1999). Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D(2) receptors. *J Pharmacol Exp Ther*, *291*(1), 409-415.

Wang, J. Q., Arora, A., Yang, L., Parelkar, N. K., Zhang, G., Liu, X., Choe, E. S., & Mao, L. (2005). Phosphorylation of AMPA receptors: mechanisms and synaptic plasticity. *Mol Neurobiol*, *32*(3), 237-249. https://doi.org/10.1385/mn:32:3:237

Wang, K. S., McClure, J. P., Alselehdar, S. K., & Kanta, V. (2015). Direct and indirect pathways of the basal ganglia: opponents or collaborators? [General Commentary]. *Frontiers in Neuroanatomy*, *9*. https://doi.org/10.3389/fnana.2015.00020

Wang, Q., Zhao, J., Brady, A. E., Feng, J., Allen, P. B., Lefkowitz, R. J., Greengard, P., & Limbird, L. E. (2004). Spinophilin blocks arrestin actions in vitro and in vivo at G protein-coupled receptors. *Science*, *304*(5679), 1940-1944. https://doi.org/10.1126/science.1098274

Waszczak, B. L., Martin, L. P., Finlay, H. E., Zahr, N., & Stellar, J. R. (2002). Effects of Individual and Concurrent Stimulation of Striatal D₁ and D₂ Dopamine Receptors on Electrophysiological and Behavioral Output from Rat Basal Ganglia. *Journal of Pharmacology and Experimental Therapeutics*, *300*(3), 850. https://doi.org/10.1124/jpet.300.3.850

Watkins, D. S., True, J. D., Mosley, A. L., & Baucum, A. J. (2019). Correction: Baucum II, Anthony J. et al. Proteomic Analysis of the Spinophilin Interactome in Rodent Striatum Following Psychostimulant Sensitization. Proteomes 2018, 6, 53. *Proteomes*, *7*(1), 7. https://doi.org/10.3390/proteomes7010007

Watkins, D. S., True, J. D., Mosley, A. L., & Baucum, A. J., 2nd. (2018). Proteomic Analysis of the Spinophilin Interactome in Rodent Striatum Following Psychostimulant Sensitization. *Proteomes*, *6*(4), 53. https://doi.org/10.3390/proteomes6040053

Welter, M. L., Burbaud, P., Fernandez-Vidal, S., Bardinet, E., Coste, J., Piallat, B., Borg, M., Besnard, S., Sauleau, P., Devaux, B., Pidoux, B., Chaynes, P., Tézenas du Montcel, S., Bastian, A., Langbour, N., Teillant, A., Haynes, W., Yelnik, J., Karachi, C., . . . French Stimulation dans Trouble Obsessionnel Compulsif Study, G. (2011). Basal ganglia dysfunction in OCD: subthalamic neuronal activity correlates with symptoms severity and predicts high-frequency stimulation efficacy. *Translational psychiatry*, *1*(5), e5-e5. https://doi.org/10.1038/tp.2011.5

Westphal, R. S., Tavalin, S. J., Lin, J. W., Alto, N. M., Fraser, I. D., Langeberg, L. K., Sheng, M., & Scott, J. D. (1999). Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science*, *285*(5424), 93-96. https://doi.org/10.1126/science.285.5424.93

Woolfrey, K. M., & Dell'Acqua, M. L. (2015). Coordination of Protein Phosphorylation and Dephosphorylation in Synaptic Plasticity. *The Journal of biological chemistry*, *290*(48), 28604-28612. https://doi.org/10.1074/jbc.R115.657262

Xiong, Q., Znamenskiy, P., & Zador, A. M. (2015). Selective corticostriatal plasticity during acquisition of an auditory discrimination task. *Nature*, *521*(7552), 348-351. https://doi.org/10.1038/nature14225

Xue, B., Chen, E. C., He, N., Jin, D.-Z., Mao, L.-M., & Wang, J. Q. (2017). Integrated regulation of AMPA glutamate receptor phosphorylation in the striatum by dopamine and acetylcholine. *Neuropharmacology*, *112*(Pt A), 57-65. https://doi.org/10.1016/j.neuropharm.2016.04.005

Yager, L. M., Garcia, A. F., Wunsch, A. M., & Ferguson, S. M. (2015). The ins and outs of the striatum: role in drug addiction. *Neuroscience*, *301*, 529-541. https://doi.org/10.1016/j.neuroscience.2015.06.033

Yan, Z., Hsieh-Wilson, L., Feng, J., Tomizawa, K., Allen, P. B., Fienberg, A. A., Nairn, A. C., & Greengard, P. (1999). Protein phosphatase 1 modulation of neostriatal AMPA channels: regulation by DARPP-32 and spinophilin. *Nat Neurosci*, *2*(1), 13-17. https://doi.org/10.1038/4516

Ye, F., & Zhang, M. (2013). Structures and target recognition modes of PDZ domains: recurring themes and emerging pictures. *Biochemical Journal*, *455*(1), 1-14. https://doi.org/10.1042/BJ20130783

Yelnik, J., Percheron, G., & François, C. (1984). A Golgi analysis of the primate globus pallidus. II. Quantitative morphology and spatial orientation of dendritic arborizations. *Journal of Comparative Neurology*, *227*(2), 200-213. https://doi.org/https://doi.org/10.1002/cne.902270206

Yger, M., & Girault, J.-A. (2011). DARPP-32, Jack of All Trades… Master of Which? [Review]. *Frontiers in Behavioral Neuroscience*, *5*. https://doi.org/10.3389/fnbeh.2011.00056

Yin, H. H., & Lovinger, D. M. (2006). Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling. *Proc Natl Acad Sci U S A*, *103*(21), 8251-8256. https://doi.org/10.1073/pnas.0510797103

Yin, H. H., Mulcare, S. P., Hilário, M. R., Clouse, E., Holloway, T., Davis, M. I., Hansson, A. C., Lovinger, D. M., & Costa, R. M. (2009). Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nat Neurosci*, *12*(3), 333-341. https://doi.org/10.1038/nn.2261

Yoest, K. E., Quigley, J. A., & Becker, J. B. (2018). Rapid effects of ovarian hormones in dorsal striatum and nucleus accumbens. *Hormones and behavior*, *104*, 119-129. https://doi.org/10.1016/j.yhbeh.2018.04.002

Zhu, J., Shang, Y., & Zhang, M. (2016). Mechanistic basis of MAGUK-organized complexes in synaptic development and signalling. *Nature Reviews Neuroscience*, *17*(4), 209-223. https://doi.org/10.1038/nrn.2016.18

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MANUSCRIPTS

Resendiz M[1], **Watkins DS[1]**, Öztürk *&* Zhou FC. (2021) . Chapter 31 – Environmental Influence of Epigenetics. In T.O. Tollefsbol (Ed.), *Handbook of Epigenetics (Third Edition) (pp.):* Academic Press. (*In Review*)

Watkins DS[1], True JD, Mosley AL, *&* Baucum AJ. (2018). Proteomic analysis of the spinophilin interactome in rodent striatum following psychostimulant sensitization. ***Proteomes,*** 6(4), 53. Doi:10.3390/proteomes6040053.

Morris CW[1], **Watkins DS[1]**, Salek AB[1], Edler MC, *&* Baucum AJ. (2018) . The association of spinophilin with disks large-associated protein 3 (SAPAP3) is regulated by metabotropic glutamate receptor (mGluR)5. ***Molecular and Cellular Neuroscience***, *90,* 60-69.

Edler MC[1], Salek AB[1], **Watkins DS[1]**, Kaur H, Morris CW, Yamamoto BK, *&* Baucum AJ. (2018) . Mechanisms regulating the association of protein phosphatase 1 with spinophilin and neurabin. *ACS Chemical Neuroscience*, 9(11), 2701-2712.

RESEARCH PRESENTATIONS

Watkins DS, Morris CW, Muñoz B, *&* Baucum AJ. (2021) Striatal Spinophilin and amphetamine sensitization. Marine Biological Laboratories SPINES. Woods Hole, MA, US.

Watkins DS, Muñoz B, *&* Baucum AJ. (2019) Cell-specific spinophilin function following psychostimulant-induced behavioral sensitization regimens. Society for Neuroscience, Chicago, IL, US.

Watkins DS, *&* Baucum AJ. (2019) Cell-specific spinophilin function following psychostimulant-induced behavioral sensitization regimens. Gordon Research Conference, Manchester, NH, US.

Watkins DS, *&* Baucum AJ. (2019) Cell-specific spinophilin function following psychostimulant-induced behavioral sensitization regimens. NIDA Scholar Professional Development Workshop. Washington, DC, US.

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Watkins DS, *&* Baucum AJ. (2018) Cell-specific spinophilin function following psychostimulant-induced behavioral sensitization regimens. Greater Indiana SFN Annual Meeting, Lafayette, IN, US.

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SOCIETY AFILIATIONS

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