DISSECTING THE CONTRIBUTION OF POTASSIUM CURRENTS TO THE HETEROGENEITY IN THE EXCITABILITY PROPERTIES OF MIDBRAIN DOPAMINE NEURON SUBPOPULATIONS

By

Rahilla A. Tarfa

B.S., University of Maryland, Baltimore County (UMBC)

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Department of Neuroscience at Brown University

Providence, Rhode Island

May, 2017
This dissertation by Rahilla A. Tarfa is accepted in its present form by the Department of Neuroscience as satisfying the dissertation requirement for the degree of Doctor of Philosophy.

Date________________           _________________________________________________

Dr. Zayd M. Khaliq, Advisor

Recommended to the Graduate Council

Date________________           _________________________________________________

Dr. Jeffery Diamond, Reader

Date________________           _________________________________________________

Dr. Aryn Gittis, Reader

Date________________           _________________________________________________

Dr. Dax Hoffman, Reader

Date________________           _________________________________________________

Dr. Diane Lipscombe, Reader

Date________________           _________________________________________________

Dr. Chris McBain, Reader

Approved by the Graduate Council

Date________________           _________________________________________________

Dr. Andrew G. Campbell
Dean of the Graduate School
Brown University
EDUCATION
Brown University/NIH-Graduate Partnership Program (GPP)
Ph.D. in Neuroscience 2011-2017

University of Maryland, Baltimore County (UMBC)
B.S. in Bioinformatics and Computational Biology 2006-2011
Minors: Chemistry, Computer Science and English Writing

RESEARCH EXPERIENCE
Research Advisor – Zayd M. Khaliq, Ph.D.

Studied subpopulations of midbrain dopamine neurons – mesocortical, mesoaccumbal and mesostriatal using patch-clamp electrophysiology, laser scanning microscopy and immunostaining techniques to assay firing properties and underlying potassium currents that contribute to the diversity in excitability of dopamine neuron subpopulations.

Showed that mesoaccumbal dopamine neurons display enhanced sensitivity to hyperpolarizing stimuli compared to mesostriatal dopamine neurons because of an atypically slowly inactivating “transient” (A-type) potassium current present within them. Carried out biophysical characterizations of the A-type potassium currents in both subpopulations, and related them to their firing behavior. Project resulted in a first-authored paper in the Journal of Neuroscience.

Showed that mesocortical dopamine neurons fire at remarkably higher evoked firing rates compared to mesoaccumbal and mesostriatal dopamine neurons, not due to differences in their D2-autoreceptors but the presence of calcium-activated potassium currents in mesoaccumbal and mesostriatal dopamine neurons. Currently investigating the differential contribution of the voltage-gated K\textsubscript{V}2 and K\textsubscript{V}3 currents in shaping the difference in high frequency firing among the dopamine neuron subpopulations. Project is still in progress.

Undergraduate Research – Biological Sciences Department, University of Maryland, Baltimore County (UMBC). 2009-2011.
Research Advisor – Thomas Cronin, Ph.D.

Using molecular biology techniques such as polymerase chain reaction (PCR), DNA quantification, genetic analysis programs, and phylogenetic trees, worked on bar-coding larval stomatopods to investigate the genetic diversity and development of these species from Lizard Island Research Station in Australia.

Submitted part of work as Independent Honors Study for UMBC Honors College.
Undergraduate Research – Biological Sciences Department, Brandeis University. Summer Internship 2009 & 2010. Funded by Short-Term Research Experience for Under-Represented Minorities (STEP-UP) under the NIH/National Institute of Diabetes, Digestive and Kidney Diseases (NIH/NIDDK)
Research Advisor – Suzanne Paradis, Ph.D.
Investigated the role of the small molecule Rem2 and the adhesion protein Cadherin-13 in inhibitory synapse development in hippocampal neurons. Performed rescue assays using primary hippocampal cultures, cDNA transfection and laser scanning microscopy.

Presented work as oral and poster presentations at the 2009 and 2010 STEP-UP Annual conferences.

Undergraduate Research – Nephrology Department, University of Maryland School of Medicine (UMB). Summer Internship 2007 & 2008. Funded by Short-Term Research Experience for Under-Represented Minorities (STEP-UP) under the NIH/National Institute of Diabetes, Digestive and Kidney Diseases (NIH/NIDDK)
Research Advisor – Moo H. Kwon, Ph.D.
Investigated blood pressure levels in mice that were wild-type for the transcription factor tonicity-responsive enhancer binding protein (TonEBP) versus mice that were haploinsufficient for TonEBP at different time courses – five and ten weeks in male mice. Learned how to ear-tag mice, genotype mice - Wild vs. Knockout for TonEBP, measure mice blood pressure, PCR, gel electrophoresis, DNA quantification and mouse kidney surgery.

Investigated how the transcriptional factor known as TonEBP (tonicity-responsive enhancer binding protein) could affect the survival rates of mouse embryonic fibroblasts (MEF). Examined for the presence of secreted proteins to see if they were responsible for the higher survival rates and less sensitivity to hypertonic stress exhibited by mice that had TonEBP present, versus the knockout mice. Employed the use of western blots, immunoblotting assays and radioactive image development.

Presented work as oral and poster presentations at the 2008 and 2009 STEP-UP Annual conferences.

PUBLICATIONS

ABSTRACTS AND CONFERENCE PRESENTATIONS
Tarfa, RA and Khaliq, ZM. Comparison of High Frequency Firing in Mesocortical, Mesoaccumbal and Mesostriatal dopamine neurons. Neuroscience 2016 - The Annual meeting for the Society for Neuroscience.
Tarfa, RA and Khaliq, ZM. Excitability and ionic Currents in midbrain dopamine subpopulations:
A-Type Potassium Currents. 11th Annual NIH Graduate Student Research Symposium 2015. 
Tarfa, RA and Khaliq, ZM. Diversity of ionic currents in VTA Dopamine Neurons. 9th Annual 
NIH Graduate Student Research Symposium 2013.

INVITED TALKS
“Excitability in subpopulations of Midbrain Dopamine Neurons”. Selected Speaker - 11th Annual 
NIH Graduate Student Research Symposium 2015.

TEACHING EXPERIENCE
Teaching Assistant – Honors 100, Introductory Honors Course of the Honors College @ UMBC.

LEADERSHIP/COMMITTEES
Undergraduate:
Elected Senator, Student Government Association (UMBC) 2007-2010. 
Senate Speaker, Student Government Association (UMBC) 2009. 
Large Group Coordinator, Intervarsity Christian Fellowship (IVCF), UMBC 2010-2011. 

Graduate:
Co-Editor, GSChronicles – Newsletter for the NIH Graduate Student Chronicles 2015-2017. 

HONORS/AWARDS/ACCOMPLISHMENTS
Secondary School:
Peak Performer of the Year Award by West African Milk Company, 2002 - N100,000 (~$900) 
Cowbell National Secondary Schools Competition, Kano State 2nd place winner, 2004

Undergraduate:
Dean’s List (Fall, 2006) and Semester Academic Honors (Fall, 2006 and Fall, 2007) 
Short-Term Research Experience for Under-Represented Minorities (STEP-UP) under the 
Emerging Researchers Network (ERN) National Science Foundation (NSF) Travel Award 
(2010)

Graduate:
NIH Fellows Award for Research Excellence (FARE) Competition, Winner (2017) ($1000) 
Associate, Neuroscience Scholars Program (NSP) – Society for Neuroscience (SfN) (2014- 
2016)
Best Poster in Neuroscience, NIH-Graduate Student Research Award ($1000) (NGRSA) (2013)

SOCIETY MEMBERSHIPS (PAST AND CURRENT)
National Society for Collegiate Scholars (NSCS) 
Golden Key International Society 
Society for Neuroscience (SfN)
ACKNOWLEDGEMENTS

There is an African adage that says: “It takes a village to raise a child”. Indeed, it took a village with me. From little seedlings that were sown in college to this momentous day, it took a troupe of mentors, family and friends to get me here - all of whom I am very grateful to.

I would like to thank my mentor – Dr. Zayd Khaliq, who took his time over the past few years imparting, teaching and growing me into a scientist. Indeed, his curiosity, enthusiasm and devotion toward science is infectious. He has modeled what a scientist and mentor should be, and for that, I am very grateful.

I am thankful to the members of my thesis committee – Drs. Diamond, Hoffman, Lipscombe and McBain for taking the time out to provide input to my project over the years, and providing professional advice and assistance along the way. I would also like to thank Dr. Gittis for serving as an external reader for my dissertation and providing feedback.

I would also like to thank my past mentors – Drs. H.M. Kwon, Thomas Cronin and Suzanne Paradis, individuals who encouraged my scientific journey in college, giving me the opportunity to explore and dig deep in lab, which propelled me to pursue a Ph.D. in Neuroscience.

I would like to thank the Neuroscience department at Brown University, and my cohort class of 2011 who created a warm and supportive community to grow professionally and as a person.

I would like to thank the members of the Khaliq lab, past and present – Ally, Travis, Richie, Rebekah, Manhua, Fabian and Paul, who provided help and encouragement during both the low and high times, and for providing a constant source of cheer in the lab.

I am grateful to the many dear friends over the past few years - Dembi, Meron, Adebimpe, Yewande, Obiageri, Hyeladzira and Akinola, to mention a few who have lent a listening ear, their time and efforts in being not only a source of encouragement but also being my family away from home. Indeed, you all made my days brighter.
I would like to thank my family – my parents, Mr. and Mrs. Ali Tarfa, and my siblings, Adams and Hannatu, whose constant encouragement and fearless love gave me the courage to venture out from our home in Kano, Nigeria and pursue an unknowable path at the time. Indeed, your trust and faith in me always kept me safe and forging ahead. To my guardian – Mr. Abu Nasara, who cared for me as his own daughter when I was without my immediate family in the U.S., I thank you for your care and devotion.

I would like to dedicate this dissertation to my grandparents – Adamu and Yejinma Tarfa, and Thalma and Mbaya Malgwi, as though it were their own.

Last but not least, I would like to thank Almighty God for fortitude for the years past and ahead.
PREFACE

Dopamine neurons have been routinely studied in the context of their broad anatomical locations in the Ventral Tegmental Area (VTA) and Substantia Nigra Compacta (SNc), despite the heterogeneity that exists in their electrophysiological profiles, biochemical markers, axonal projections and behavioral correlates. Anatomical, electrophysiological and behavioral studies support the categorization of dopamine neurons as subpopulations based on their axonal projections, as these subpopulations also display functional heterogeneity. The overall goal of this dissertation is to investigate the diversity in the excitability properties among midbrain dopamine neuron subpopulations, and provide mechanistic insights into this diversity. I ask questions on how two major states of excitability – pauses (Chapter 2) and high frequency firing (chapter 3), are different among the dopamine neuron subpopulations. To this end, I provide detailed characterizations of subthreshold and supra-threshold potassium currents that contribute to the differences in the excitability of the dopamine neuron subpopulations.

For my first project (chapter 2), I investigated and characterized cellular pauses among accumbal-projecting (mesoaccumbal) and striatal-projecting (mesostriatal) dopamine neurons. Notable differences have been observed in the pause duration among the dopamine neurons with little insight into the intrinsic properties that underlie such observations. I find that hyperpolarization initiated pauses, even within the physiological voltage range, are longer among mesoaccumbal as compared to mesostriatal dopamine neurons. Such pauses are strongly shaped by both the strength and duration of the current injections, raising questions of the nature of inhibitory inputs that would initiate such pauses in vivo. Interestingly, I found that hyperpolarization evoked cellular pauses (rebound delay) relates to the slope of the interspike interval, denoting its possible use as a predictive tool for the neuron’s response to inhibitory inputs. I then asked what ionic currents underlie the differences in cellular pauses among the mesoaccumbal and mesostriatal dopamine neurons. I examined for the presence of A-type potassium currents and hyperpolarization-activated cationic currents (H-currents), two of the
known subthreshold currents among the dopamine neurons. I found that mesoaccumbal dopamine neurons expressed A-type potassium currents that inactivate on a slower time scale than has been previously shown among dopamine neurons. In contrast, mesostriatal dopamine neurons displayed A-type potassium currents that inactivate on a faster timescale as had been previously observed. For H-currents, I found that mesostriatal dopamine neurons displayed a larger amplitude of the current compared to mesoaccumbal dopamine neurons. We found that the A-type potassium currents could facilitate synaptically evoked GABAergic pauses as well those induced by GABA uncaging. Finally, using computational modelling, we showed that the inactivation kinetics of $I_A$ is an overwhelming determinant of the duration of the pause, even in the presence of large amplitudes of H-currents and T-type calcium currents.

For my second project (chapter 3), I turned my attention to high frequency firing among the mesocortical, mesoaccumbal and mesostriatal dopamine neurons, with the goal of understanding the potassium currents that contribute to these differences. I find that the proposed idea that differences in D2 receptor currents contributes to the differences in high frequency firing is not necessarily the case. Instead, the difference in high frequency firing among the dopamine neuron subpopulations can be attributed to differences in their intrinsic ionic currents. I find that the presence of SK currents among mesoaccumbal and mesostriatal dopamine neurons limits their high frequency firing. The presence of fast repolarization rates and narrower spikes among the mesocortical dopamine neurons indicate the possible contribution of high-threshold potassium currents. Using action potentials from previously recorded mesocortical and SNc dopamine neurons, I recorded spike elicited interspike-interval currents among mesocortical and SNc dopamine neurons via action potential clamp. I examined the contribution of $K_{V2}$ and $K_{V3}$ currents to spike evoked interspike interval currents during high frequency. I found that while both Guangitoxin (GTX) ($K_{V2}$ blocker) sensitive ($K_{V2}$) and TEA ($K_{V3}$ blocker) sensitive currents were present in both mesocortical and SNc dopamine neurons, $K_{V2}$ currents made up a larger proportion, with no indication of differential amplitudes among the
dopamine subpopulations. However, the GTX sensitive currents revealed a unique build-up that was present during high frequency firing and slower in the mesocortical as compared to the SNC dopamine neurons. As such, the differential presence of SK and differential build-up kinetics of Kv2 currents are strong underlying mechanisms that could explain the differential excitability properties among the mesocortical, mesoaccumbal and mesostriatal dopamine neuron subpopulations.
# TABLE OF CONTENTS

## CHAPTER 1

1.1 HISTORICAL CONTEXT OF MIDBRAIN DOPAMINE NEURONS. .................................................. 1

1.2 MIDBRAIN DOPAMINE NEURONS: STUDIES ON BEHAVIORAL CORRELATES, FUNCTION, AND DISEASE.................................................................................................................. 2

1.3 GENERAL OVERVIEW OF INPUTS TO AND OUTPUTS OF MIDBRAIN DOPAMINE NEURONS. .......... 4

1.4 CLASSIFICATION OF MIDBRAIN DOPAMINE NEURONS INTO SUBPOPULATIONS: INSIGHTS FROM BEHAVIOR AND NEUROCIRCUITRY ................................................................. 10

1.5 EXCITABILITY OF MIDBRAIN DOPAMINE NEURONS – GENERAL OVERVIEW AND FUNCTIONAL ASPECTS .......................................................................................................................... 13

1.6 IONIC CONDUCTANCES THAT UNDERLIE THE HETEROGENEITY IN THE EXCITABILITY OF MIDBRAIN DOPAMINE NEURON SUBPOPULATIONS ............................................................................... 17

1.6.1 PACEMAKING IN SNC vs VTA DOPAMINE NEURONS – ........................................................................ 17

1.6.2. BURST FIRING OR HIGH FREQUENCY FIRING IN SNc vs VTA DOPAMINE NEURONS – .................. 22

1.6.3 PAUSES IN SNc VS VTA DOPAMINE NEURONS .................................................................................. 25

1.7 SUMMARY ....................................................................................................................................... 28

## CHAPTER 2

2.1 ABSTRACT ..................................................................................................................................... 32

2.2 INTRODUCTION ............................................................................................................................ 33

2.3 METHODS .................................................................................................................................... 34

2.4 RESULTS ...................................................................................................................................... 37

2.4.1 Similar post-burst pauses but larger rebound delays in mesoaccumbal versus nigrostriatal dopaminergic neurons .......................................................................................................................... 43

2.4.2 Development of rebound delays evoked by a range of brief duration hyperpolarizations ........... 45

2.4.3 Effect of subthreshold inhibitory current pulses on spike timing during pacemaking ............. 46

2.4.4 Hyperpolarization-evoked spiking delays are lengthened by a subclass of subthreshold potassium current (I<sub>h</sub>) ..................................................................................................................... 48

2.4.5 GABA-evoked pauses are prolonged by recruitment of A-type potassium currents ................ 49

2.4.6 Comparison of inactivation kinetics, voltage dependence, and recovery of A-type potassium currents in mesoaccumbal and nigrostriatal neurons ................................................................. 50

2.4.7 Comparison of I<sub>h</sub> and sag potentials in mesoaccumbal and nigrostriatal neurons ................. 53

2.4.8 Correlating A-type potassium current kinetics and I<sub>h</sub> amplitude to the duration of rebound delay in mesoaccumbal and nigrostriatal dopamine neurons ................................................. 54

2.4.9 Testing the relative contribution of I<sub>h</sub> kinetics and I<sub>h</sub> amplitude to rebound delay in a computational dopamine neuron model .......................................................................................................................... 54

2.5 DISCUSSION ................................................................................................................................. 56

2.5.1 Heterogeneity in I<sub>h</sub> inactivation kinetics - molecular mechanism ................................................... 57

2.5.2 Diversity of pauses and underlying mechanisms in midbrain dopamine neurons .................. 59

2.5.3 Functional Significance ............................................................................................................. 59

## CHAPTER 3 ................................................................................................................................. 83

3.1 ABSTRACT .................................................................................................................................. 84

3.2 INTRODUCTION .......................................................................................................................... 85

3.3 METHODS ................................................................................................................................. 88

3.4 RESULTS ................................................................................................................................. 92
3.4.1 Higher firing rates among the Mesocortical dopamine subpopulation as compared to the Mesoaccumbal and Mesostriatal dopamine subpopulation. ................................................................. 92
3.4.2 Differences in the D2 auto-receptor currents among the dopamine neuron subpopulations do not limit high frequency firing. ................................................................. 95
3.4.3 Distinct difference in a high frequency evoked after-hyperpolarization (eAHP) is mediated by the small-conductance calcium-activated potassium (SK) current. ........................................... 96
3.4.4 The contribution of high threshold delay rectifier potassium (K+) currents to large step depolarizations among dopamine neuron subpopulations. ...................................... 98
3.4.5 Examining the contribution of high threshold delayed rectifier potassium (K+) currents to spike evoked interspike interval currents in dopamine neurons. ..................................... 100
3.4.6 K+ currents accumulate differentially during high frequency firing in mesocortical and SNc dopamine neurons. ...................................................................................................... 103
3.5 DISCUSSION .................................................................................................................. 105
3.5.1 Differences in D2-receptor mediated currents do not underlie high frequency firing. .... 105
3.5.2 SK currents contribute to differences in high frequency firing among the dopamine neuron subpopulations, while BK currents do not ................................................................. 106
3.5.3 K+ currents among the mesocortical and SNc dopamine neurons. ........................... 108
3.5.4 K+ currents among the mesocortical and SNc dopamine neurons. ............................ 110

CHAPTER 4 ........................................................................................................................... 130
4.1 SUMMARY ..................................................................................................................... 131
4.2 FURTHER ELUCIDATING THE UNDERPINNINGS OF PAUSES IN MIDBRAIN DOPAMINE NEURON SUBPOPULATIONS ........................................................................ 134
4.3 FURTHER UNCOVERING THE ELEMENTS OF DIFFERENTIAL FIRING AMONG THE MIDBRAIN DOPAMINE NEURON SUBPOPULATIONS, AND TECHNICAL CONSIDERATIONS ........................................ 137

Bibliography: ...................................................................................................................... 140
TABLE OF FIGURES

Figure 1-1: A topographical illustration of the midbrain dopamine neurons in the SNc and the VTA .................................................. 30
Figure 1-2 The somatodendritic activation of D2 autoreceptors elicits an inhibitory post synaptic current that hyperpolarizes dopamine neurons and results in their quiescence (pause) ........................................... 31
Figure 2-1 Retrograde labelling of mesoaccumbal and nigrostriatal dopamine neurons .............................................................................................. 62
Figure 2-2 Comparison of post-burst pauses and rebound delays in mesoaccumbal and nigrostriatal dopamine subpopulations ........................................... 64
Figure 2-3 Time-dependent development of the rebound delay ........................................... 66
Figure 2-4 Effect of short duration hyperpolarizations in mesoaccumbal and nigrostriatal dopamine neurons ................................................................. 68
Figure 2-5 AmmTX3, a specific blocker of Kv4 subunits, abolishes rebound pauses of all durations in mesoaccumbal and nigrostriatal dopamine neurons .......... 70
Figure 2-6 Pauses evoked by inhibitory synaptic stimulation and GABA uncaging reduced by AmmTX3 block of A-type potassium currents .................................................................................. 72
Figure 2-7 Biophysical properties of A-type potassium currents midbrain dopamine neurons ................................................................. 74
Figure 2-8 Recovery from inactivation of A-type potassium currents in mesoaccumbal and mesostriatal dopamine neurons ......................................................... 76
Figure 2-9 Biophysical properties of H-current in mesoaccumbal and nigrostriatal subpopulations ......................................................................................... 78
Figure 2-10 Correlation of inactivation kinetics of IA and the amplitude of IH with rebound properties in mesoaccumbal and nigrostriatal dopamine neurons .......... 80
Figure 2-11 Computational model testing the relative contribution of IA, IH, and IF to the rebound delay and GABA-A-evoked pauses in firing ........................................ 82
Figure 3-1 Midbrain dopamine neuron subpopulations exhibit different firing frequencies ................................................................................................. 113
Figure 3-2 Comparison of spike features among the mesocortical, mesoaccumbal and mesostriatal dopamine neurons .............................................................. 115
Figure 3-3 Lack of effect of the D2 antagonist, Sulpiride, and GIRK blocker, Ba2+ on mesocortical and mesoaccumbal dopamine neuron high frequency firing .......... 117
Figure 3-4 Midbrain dopamine neuron subpopulations exhibit heterogeneity in an evoked after-hyperpolarization potential (EAHP) ........................................... 119
Figure 3-5 SK currents underlie the EAHP, and limit high frequency firing in mesoaccumbal dopamine neurons ................................................................. 121
Figure 3-6 Comparing Kv2 and Kv3 currents in response to step depolarizations in mesocortical, mesoaccumbal and SNc dopamine neurons .................................. 123
Figure 3-7 Comparing spike evoked interspike interval Kv2 and Kv3 currents in mesocortical and SNc dopamine neurons in response to a waveform previously recorded in a mesocortical dopamine neuron .......................................................... 125
Figure 3-8 Comparing spike evoked interspike interval Kv2 and Kv3 currents in mesocortical and SNc dopamine neurons in response to a waveform previously recorded in a SNc dopamine neuron .......................................................... 127
Figure 3-9 Build-up of the spike evoked interspike interval Kv2 currents in mesocortical, mesoaccumbal and SNc dopamine neurons .................................................. 129
CHAPTER 1

INTRODUCTION
1.1 HISTORICAL CONTEXT OF MIDBRAIN DOPAMINE NEURONS.

Embedded at the base of the mesencephalon are dopamine neurons, a group of essential neurons that encode for a surprisingly wide range of cardinal behaviors. In a landmark paper by Carlsson, dopamine neurons were first characterized alongside other monoaminergic neurons using the Falck-Hillarp method, a formaldehyde visualization technique (Carlsson and Waldeck, 1958; Carlsson, 1959). The explosive interest in dopamine neurons in the field of neuroscience subsequently grew with their implication as the group of neurons that die in Parkinson’s disease (Hornykiewicz, 1966; Lees et al., 2015). After decades in the advancement of sophisticated neuroscience tools used to investigate dopamine neurons, we now know that dopamine neurons are more complex than their identity as monoaminergic neurons that die in Parkinson’s disease. Indeed, midbrain dopamine neurons are integrators of a wide variety of complex sensory and motor stimuli that enable them to encode a range of essential behaviors.

The decades that followed the discovery and identification of the monoaminergic neurons, particularly the ‘70s and ‘80s, saw an increase in experiments that sought to understand just how different brain regions affected animal behavior. Behavioral studies into the role of dopamine neurons set a pioneering blaze in the field. The first discovered role of the dopamine neurons is the regulation of sensory-motor integration. Due to the severity of the symptoms it causes, it is also the most well-known role of the dopamine neurons. Its role in sensory-motor integration was uncovered from classic case and lesion studies that demonstrated that the debilitating movement disorder, Parkinson’s disease, results from the degeneration of SNc dopamine neurons (Hornykiewicz, 1966; Ungerstedt, 1968; Uretsky and Iversen, 1970; Ljungberg and Ungerstedt, 1976; Bjorklund and Dunnett, 2007b; Lees et al., 2015). Incidentally, this initial finding established the regulation of movement as the ubiquitous function of dopamine neurons. Concurrently, a series of subsequent experiments demonstrated that rodents would self-administer cocaine and amphetamine (‘reward-seeking’), and that the intervals of administration, and in another case self-stimulation, would decrease when rodents
were treated with dopamine receptor antagonists (neuroleptics) (Yokel and Wise, 1975, 1976; Fouriezos et al., 1978). However, these findings were incompatible with the universally accepted sole role of dopamine neurons in the regulation of movement.

The ineffectiveness of dopamine antagonists (neuroleptics) to cause complete motor immobilization but rather a decrease in ‘reward-seeking’ was not well understood until two types of anatomical studies were carried out. The first were a series of experiments aimed at mapping the boundaries of the brain regions that resulted in rodent self-stimulation. These experiments showed that the substantia nigra compacta (SNC) and the ventral tegmental area (VTA), where the dopamine neurons are located, formed these rodent ‘self-stimulation’ boundaries (Corbett and Wise, 1980). The second set of studies focused on delineating the brain regions that received dopaminergic terminals and as such, played a role in affecting the prior self-stimulation results observed in rodents and non-human primates. One experiment demonstrated that neuroleptics selectively injected into the nucleus accumbens of rodents resulted in marked reductions in the self-stimulation by these rodents (Mogenson et al., 1979). Another experiment demonstrated that the depletion of dopamine in the prefrontal cortex resulted in a delayed cortical function during a spatial performance task (Brozoski et al., 1979). Similarly, the use of dopamine antagonists in the caudate/putamen versus the prefrontal cortex, showed a differential response with self-stimulation in the rodent VTA area, with the caudate/putamen as the source (Mora et al., 1977). Similar results were also observed in non-human primates (Phillips et al., 1979). As such, these ‘self-stimulation boundaries’ and ‘self-stimulation sites’ experiments demonstrated that there existed a heterogeneity in functional role of dopamine neurons.

These set of elegant behavioral experiments were also supported with insights from anatomical tracing studies. Initial mappings of the SNC terminals demonstrated their large terminal arborizations in the striatum (Anden et al., 1964; Dahlstroem and Fuxe, 1964). Subsequent mappings of dopamine neurons from the VTA found a vastly more diverse range of
terminals in other brain regions that included the amygdala, septum, prefrontal cortex, piriform
cortex, cingulate cortex, nucleus accumbens and olfactory tubercle among others (Ungerstedt,
1971; Fallon et al., 1978; Fallon and Moore, 1978a, b; Oades and Halliday, 1987). Together,
these behavioral and anatomical experiments were pivotal in establishing the first instance of
dual functionality among the dopamine neurons. On the one hand, the SNc dopamine neurons
form the nigrostriatal pathway which project to the striatum, degenerate in Parkinson’s disease
and function in sensory-motor integration. On the other hand, the VTA dopamine neurons
form the mesocorticolimbic pathway which project to cortico-limbic regions, and are more involved in
the reward circuitry or the ‘pleasure center’ as the original self-stimulation scientists, Olds and
Milner, called it (Olds and Milner, 1954). As such, these early neuroanatomical and behavioral
findings set up the dichotomous classification of the midbrain dopamine neurons into the broad
sub-groups of the SNc and VTA based on location and their roles in locomotion and reward
respectively.

1.2 MIDBRAIN DOPAMINE NEURONS: STUDIES ON BEHAVIORAL CORRELATES,
FUNCTION, AND DISEASE.

The advent of in vivo electrophysiology made it possible to directly map the neural
substrates that encode for environmental cues. As such, initial functional studies on the reward
circuitry sought to directly identify the “retina of the reward system” (Schultz, 2007). Dopamine
neurons were identified as the primary candidate due to the following pieces of evidence from
behavioral studies: i) a reduction in reward-seeking behavior in rodents given neuroleptics
(Fouriezos and Wise, 1976; Corbett and Wise, 1980; Prado-Alcala et al., 1984; Prado-Alcala
and Wise, 1984), ii) facilitation of self-stimulation in response to cocaine and amphetamine
(Yokel and Wise, 1976; Robertson and Mogenson, 1979) and iii) brain regions that received
dopamine neuron terminals were the best sites to elicit self-stimulation in rodents (Mora et al.,
1977; Prado-Alcala et al., 1984; Prado-Alcala and Wise, 1984). The precise role of dopamine
neurons in reward was mapped out using single-unit recordings from awake-behaving non-
human primates during reward-related task paradigms. In a series of several experiments, Schultz and colleagues demonstrated that dopamine neurons: i) did not burst in response to purely sensory stimuli or motor responses (Schultz, 1986), ii) burst in response to appetitive stimuli (Schultz and Romo, 1992), iii) burst to stimuli predicting reward during learning and unexpected stimuli (Ljungberg et al., 1992; Romo et al., 1992), iv) depress their tonic activity in the absence of expected reward (Schultz et al., 1993) and v) do not respond to aversive stimuli (Mirenowicz and Schultz, 1996). As such, dopamine neurons will burst in response to unpredicted reward and when the task is being learnt (that is, when the conditioned stimulus is being paired with reward). However, after learning has taken place, they will only respond to the conditioned stimulus and not the reward itself (Schultz et al., 1997). As a result of these findings, dopamine neurons (both SNc and VTA) became conventionally labeled as the “reward neurons”, as they quantitatively encode for reward and error prediction (Schultz, 2007).

One conclusion that emerged from the above set of elegantly designed studies was the implication that dopamine neurons are ubiquitous in function as positive reward-error prediction ‘encoders’ (Schultz et al., 1997). However, this observation is incompatible with earlier findings that demonstrate the additional involvement of dopamine neurons in non-reward functions like sensory-motor integration (see section above). Recent work by Matsumoto and Hikosaka has provided evidence that this convention of homogeneous dopamine neuron functionality only applies to a subset of dopamine neurons (Matsumoto and Hikosaka, 2009). Using in vivo electrophysiology functional studies in awake behaving non-human primates, in an unconditioned task, they demonstrated that a fraction of dopamine neurons burst in response to: i) a reward stimulus and were inhibited by the aversive stimulus, and ii) both rewarding and aversive stimuli. As such, they found a new group of dopamine neurons that were dissimilar to the classic dopamine response (excited by unconditioned reward and inhibited by aversive stimuli) (Mirenowicz and Schultz, 1996; Matsumoto and Hikosaka, 2009). These newly identified aversive-excited neurons are thought to encode motivational salience as the neurons’ response
correlated with latency of the monkey’s gaze shift to a conditioned stimulus, but only after it has been paired with a reward or aversive stimulus. Additionally, they found that these neurons were differentially located, with the aversive-excited neurons in the dorsolateral midbrain and the aversive-inhibited (classic dopamine response) in the ventromedial midbrain (Matsumoto and Hikosaka, 2009). Interestingly, similar findings had been demonstrated decades ago in anesthetized rodents wherein two different responses were observed among SNc dopamine neurons – those that were excited by a pinched tail, and others that were inhibited by the same tail pinch (Chiodo et al., 1980). These studies were among the first to directly shed light on the differential output responses that dopamine neurons display in response to environmental cues. They also bring up the idea of the heterogeneity of behaviors that dopamine neurons support (Bromberg-Martin et al., 2010). As such, aside from reward-error prediction, response to aversive stimuli and sensory-motor integration, dopamine neurons have been shown to encode for motivation (Berridge and Robinson, 1998; Wise, 2008), reinforcement (Cardinal, 2006), goal-directed behaviors (Cheer et al., 2007), processing of noxious stimuli (Brischoux et al., 2009), and stress (Valenti et al., 2011).

In addition to research on the role of dopamine neurons in health, human case and clinical studies have been helpful in portraying the diversity of roles that dopamine neurons play. Clinical doses of a range of neuroleptic drugs used to treat schizophrenia were shown to block the release of dopamine in rodent brain slices (Seeman et al., 1975; Seeman and Lee, 1975), thus implicating dopamine neurons in this psychiatric disorder. A reduction in indicators of dopamine synaptic markers was seen in an imaging study of human patients with ADHD versus control subjects (Volkow et al., 2009). During addiction, the dopamine reward system is hijacked by drugs of abuse (Wise, 1996). Depression has been demonstrated to be a common symptom among Parkinson’s patients (Gotham et al., 1986), leading to experiments in rodent models that show the direct involvement of the dopamine neural pathways in depression-related behaviors (Chaudhury et al., 2013; Tye et al., 2013; Friedman et al., 2014).
1.3 GENERAL OVERVIEW OF INPUTS TO AND OUTPUTS OF MIDBRAIN Dopamine Neurons.

The heterogeneity of dopamine neuron involvement in mediating different behaviors and diseases begs the question of needed insights into the underlying midbrain dopamine neuron circuitry. What are the inputs to and outputs of these clustered group of neurons in the midbrain that mediate a wide range of essential behaviors? Beginning with fluorescence studies aimed at detecting dopamine neuron terminals in different brain regions, to the latest viral-genetic tracing techniques that even combine gene profiling, midbrain dopamine neuron circuitry has been studied with great detail. As stated earlier, the SNc and VTA are the two major locations of the dopamine neurons in the midbrain, and even though we now think of them as separate areas, the neurons contained within each region are indeed contiguous, which led early neuroanatomists staining for dopamine neurons to portray the SNc-VTA as a single nucleus (Fallon and Moore, 1978a). The substantia nigra pars compacta (SNc) is a long sheet of densely packed dopamine neurons located in the mesencephalon (Moore and Bloom, 1978). Their neurons are bordered by the substantia nigra pars reticulata (SNr), a large almond-shaped GABAergic nucleus, the output of the basal ganglia (Zhou and Lee, 2011). On the other hand, the VTA is a roughly semicircular region comprised of heterogeneous neuron types consisting of dopaminergic, glutamatergic, and GABAergic neurons which lay close to the midline, and at the base of the mesencephalon (Oades and Halliday, 1987). The exact composition of the VTA somewhat differs in the literature, with the reported range as approximately 60-65% dopaminergic (Swanson, 1982), 2-3% glutamatergic (Nair-Roberts et al., 2008), and 30% GABAergic (Swanson, 1982; Sesack and Grace, 2010; Morales and Margolis, 2017).

Initial studies on the dopamine neuron circuitry were aimed at uncovering the neural pathways of the nigrostriatal dopamine neurons. Terminals of SNc dopamine neurons projections were shown to arborize in the striatum (Dahlstroem and Fuxe, 1964). Further work described a topographical organization of the projections of the SNc and VTA: ventral neurons
of the SNC and VTA projected to dorsal structures such as the striatum, septum and nucleus accumbens, while dorsal neurons of the SNC and VTA projected to the ventral structures such as the olfactory tubercle and amygdala (Fallon and Moore, 1978a). Fallon and Moore promoted the view of grouping the SNC and VTA dopamine neurons as a single nucleus. Part of this may have been due to a drawback of their study, which was the lack of specificity in the neuroanatomical tracers used. Overlapping injection sites between the SNC and the VTA may have given the impression of shared brain projection regions between the two groups of dopamine neurons. Subsequent experiments demonstrated that the SNC and VTA display different projection patterns. A tracing study that selectively studied the VTA demonstrated their projections to the amygdala, lateral hypothalamus, entorhinal cortex, nucleus accumbens, prefrontal cortex and lateral septal nucleus (Swanson, 1982), which was further confirmed by others (Phillipson, 1979; Oades and Halliday, 1987).

Inputs to the SNC and the VTA were also mapped during this time, although with similar limitations of non-specificity during the tracing method that could affect various fiber passages. Several early studies mapped out the specific inputs to the SNC to include the nucleus accumbens/ventral striatum (Swanson and Cwan, 1975), central nucleus of the amygdala (Bunney and Aghajanian, 1976), lateral preoptic and hypothalamus areas (Nauta et al., 1978), subthalamic nucleus (Nauta and Cole, 1978) and the pedunculopontine nucleus (Graybiel, 1977) (Graybiel and Ragsdale, 1979). With regards to the VTA, one observation that emerged during these anatomical studies was that different cell groups (based on brain regions) innervated different parts of the VTA. The anterior limbic cortex and ventromedial hypothalamus project to the medial VTA, which is where VTA neurons that project to the septum or habenula are located (Swanson, 1982; Oades and Halliday, 1987). The lateral septum and medical preoptic area are also specific inputs that innervate the rostroventral VTA (Swanson, 1982). Other inputs that innervate the VTA arrive from the nucleus accumbens, preoptic nucleus and lateral hypothalamus (Swanson, 1982). As such, these studies were among the first to
demonstrate the differential innervation and projection pattern of the VTA and SNc.

However, recent advances in neuroanatomical tracing methods that make use of genetic-viral tracing has enabled the element of dopamine neuron fiber specificity to be ensured. Specifically, the monosynaptic inputs of the SNc and the VTA dopamine neurons have been mapped out using the combination of the cre-lox mice and AAV viral systems, allowing for spatial resolution of whole-brain inputs onto genetically defined groups of dopamine neurons (Watabe-Uchida et al., 2012). The sensitivity of their method in this paper highlighted inputs onto dopamine neurons that had been previously overlooked. For example, in SNc neurons, dense inputs were found from the primary (M1) and secondary (M2) motor and somatosensory cortices, compared to the VTA which only received major cortical input from the orbitofrontal cortex (Watabe-Uchida et al., 2012). They also demonstrated that the dorsal raphe and the striatum were found to project to both the SNc and the VTA, with heavier innervation from the dorsal raphe to the VTA. Their work also confirmed previous results that depicted the preferential projection of the peduncular pontine nucleus to the SNc and the laterodorsal tegmental nucleus to the VTA respectively. Interestingly, in contrast to previous studies that found heavy inputs from the septum and medial lateral habenula to the dopamine neurons, they found much weaker innervations, pointing out that previous results had been impacted by non-specific labelling of non-dopaminergic neurons. Similarly, while previous work had demonstrated sparse labeling of the subthalamic inputs to the dopamine neurons, they found that these inputs were some of the strongest onto the SNc dopamine neurons. Overall, they found distinct excitatory inputs onto the SNc and the VTA, which together with prior neuroanatomical evidence, complements results from behavioral studies that demonstrate different functions of the SNc and the VTA.

The significance of these brain-wide inputs onto the dopamine neurons as well as their specific projection patterns were not fully understood until subsequent experiments were carried out that demonstrated the involvement of the dopamine neurons in different circuits. As such, for
certain behaviors like reward, locomotion and aversion, their circuitry could be specifically delineated. Together with the inputs that directly projected onto the dopamine neurons, the dopamine neurons themselves and the brain regions that they project to, dopamine neurons began to be classified as subpopulations, as they function as discrete groups mediating different aspects of behavior.

1.4 CLASSIFICATION OF MIDBRAIN DOPAMINE NEURONS INTO SUBPOPULATIONS: INSIGHTS FROM BEHAVIOR AND NEUROCIRCUITRY.

The observations of different behavioral functions of the SNc and VTA dopamine neurons has elicited the idea of dopamine neurons participating in disparate brain circuits. However, with the knowledge garnered from neuroanatomical tracing studies that demonstrated the myriad of inputs that the midbrain dopamine neurons receive, it was essential to understand the dopamine neuron circuitry in the context of the inputs they receive, their projection pattern and the specific behaviors they mediate. To test this idea, recent studies have made use of in vivo optogenetic tools to stimulate known dopaminergic pathways. These studies have led to the direct demonstration that specific groups of dopamine neurons do modulate certain aspects of essential behavior. While the nigrostriatal dopamine neuron pathway has been implicated in playing a role in modulating movement, in vivo studies have further demonstrated that these neurons signal the start and termination in a sequence of actions (Costa, 2007; Schultz, 2007; Jin and Costa, 2010). Similarly, using GCamp6, fiber photometry, and optogenetics, a recent study demonstrated that dorsal projecting dopamine neurons respond to movement, and their activation is enough to trigger natural locomotion (Howe and Dombeck, 2016). Other experiments have also highlighted the involvement of dopamine neurons in rewarding behaviors. Selective optogenetic stimulation of the VTA dopamine neurons elicits reinforcement behavior (Adamantidis et al., 2011), and specifically, among the VTA dopamine neurons that project to the nucleus accumbens, conditioned place preference was developed (Lammel et al., 2012; Lammel et al., 2014). Interestingly, these same tools have also highlighted the role of
dopamine neurons in social behaviors. Selective continuous optogenetic stimulation of the VTA dopaminergic projections to the nucleus accumbens increases social interaction time among mice previously shown to be susceptible to social defeat, a phenotype of depression among rodents (Friedman et al., 2014). Further work has shown that depression-like behavior can be bi-directionally modulated - activation of VTA dopaminergic projections to the nucleus accumbens can induce stress, while inhibiting this pathway can induce resilience (Chaudhury et al., 2013). Interestingly, similar work probing the question of bi-directional modulation of the VTA to nucleus accumbens pathway yielded results that were opposite to that of Chaudhury et al (Tye et al., 2013), differences which might be explained by the possibility that this pathway is sensitive to behavioral paradigms used to induce social defeat, stress and depression-like behavior in rodents. With regards to the other dopaminergic pathways, projections from the VTA to the amygdala play a role in emotional associations during learning (Phillips et al., 2010; Tye et al., 2010). And, projections from the VTA to the prefrontal cortex have been implicated in eliciting conditioned place aversion (Lammel et al., 2011) as well as modulating susceptibility to social defeat/depression in rodents (Chaudhury et al., 2013; Tye et al., 2013). These behavioral correlates of the dopamine neuron pathways are direct evidence of their involvement in disparate brain circuits and add to the growing body of evidence that support the examination of dopamine neurons in the context of their projection pattern as subpopulations.

With a growing appreciation of the distinct functionality that each dopaminergic pathway displays, studies on the functional (Lammel et al., 2012) and anatomical (Beier et al., 2015) inputs onto the different dopamine neuron subpopulations have emerged. Lammel and colleagues demonstrated that the lateral VTA dopamine neurons that project their axons to the lateral shell of the nucleus accumbens (also called mesoaccumbal) selectively receive inputs from the laterodorsal tegmental area, while the medial VTA which project their axons to the prefrontal cortex (also called mesocortical), selectively receive inputs from the lateral habenula (Lammel et al., 2012). These observations have prompted the idea of completely discrete and
selective inputs onto the dopamine neuron subpopulations that support their participation in
different brain circuits and hence the myriad of heterogeneous behaviors they support.
However, Beier and colleagues have demonstrated a more complex picture of the circuitry of the
dopamine neuron subpopulations. Rather than completely discrete inputs, they show
qualitatively overlapping inputs from roughly 22 brain regions onto the prefrontal, nucleus
accumbal and amygdala projecting dopamine neurons (Beier et al., 2015). Even so, when
examined quantitatively, the inputs to the dopamine neuron subpopulations are highly biased
when examined in the context of their projection patterns. For example, lateral accumbal
projecting dopamine neurons quantitatively receive more input from the anterior cortex and
striatum as compared to the medial accumbal projecting dopamine neurons, which receive more
inputs from the dorsal raphe. They also found that the prefrontal cortex innervates not just
prefrontal projecting dopamine neurons as previous results had shown (Carr and Sesack,
2000), but also innervate the nucleus accumbal projecting dopamine neurons as well.
Interestingly, they also found inputs from the lateral habenula that innervate the dopamine
neurons that project to the lateral nucleus accumbens, albeit weak. Previous work had
demonstrated that the lateral habenula did not innervate the dopamine neurons that project to
the lateral nucleus accumbens, most likely from a combination of the limitation of the technique
used in the paper and the fact that these group of neurons are anatomically located more
ventrally that initially thought (Lammel et al., 2008; Lammel et al., 2012).

Additionally, a recent paper (Lerner et al., 2015) has also examined the inputs onto the
SNC dopamine neurons in an attempt to understand the idea that there exists two distinctly
functional SNC dopamine neuron subpopulations that differentially mediate aversive and
rewarding environmental cues (Chiodo et al., 1980; Matsumoto and Hikosaka, 2009). Indeed,
Lerner and colleagues found a strong preference of a reciprocal relationship between the striatal
projection region of the SNC dopamine neurons and the SNC dopamine neurons themselves. As
such, dorsolateral striatum projecting SNC dopamine neurons received strong inputs from the
dorsolateral stratum and the caudal tail of the striatum, as compared to the dorsomedial projecting dopamine neurons that received strong inputs from the dorsomedial striatum and the nucleus accumbens (Lerner et al., 2015). Using fiber optometry to record activity in GCamp6f expressing mice, they recorded the activity of dorsolateral and dorsomedial striatal projecting dopamine neurons in response to either conditioned rewarding or aversive stimuli. Their work demonstrated that both dorsolateral and dorsomedial striatal projecting dopamine neurons responded with similar levels of activity to rewarding stimuli. Interestingly, in response to aversive stimuli, dorsolateral projecting SNc dopamine neurons showed an increase in activity while dorsomedial projecting SNc dopamine neurons showed a decrease. As such, their work adds credence to the idea that even the once-thought-to-be-homogenous nigrostriatal subpopulation is indeed heterogeneous as well.

In conclusion, the neurocircuitry of the dopamine neuron subpopulations provides strong evidence that subpopulations of dopamine neurons do indeed participate in different brain circuits, with their quantifiably discrete inputs and outputs serving as the scaffold. Justifiably so, it would be also reasonable to expect an element of heterogeneity to be present at the cellular level of the dopamine neuron subpopulations.

1.5 EXCITABILITY OF MIDBRAIN DOPAMINE NEURONS – GENERAL OVERVIEW AND FUNCTIONAL ASPECTS.

The first in vivo recordings from dopamine neurons demonstrated that they exhibit two major modes of firing - tonic and phasic. In the tonic firing mode, dopamine neurons fire slow rhythmic action potentials at an average rate of 4.5 Hz within a range of 1 - 9 Hz (Grace and Bunney, 1984b, a; Marinelli et al., 2006). The tonic firing of dopamine neurons is generally thought to contribute to the tonic, extra-synaptic levels of dopamine necessary for maintaining the dopaminergic tone to the target brain regions (Gonon, 1988; Floresco et al., 2003; Grace, 2016). Recordings from in vitro midbrain brain slice preparations containing dopamine neurons demonstrates that they also fire tonically (otherwise referred to as pacemaking) around the
same average rate and range as seen *in vivo*. Their spikes occur with a low coefficient of variation, with an average broad action potential duration of 2 - 4 ms (Marinelli et al., 2006; Khaliq and Bean, 2008, 2010). Evidence from both *in vivo* and *in vitro* preparations demonstrate that dopamine neurons can fire at higher rates than previously reported depending on where they send their axonal projections (Chiodo et al., 1984; Margolis et al., 2008; Lammel et al., 2011; Morikawa and Paladini, 2011). Chidodo and colleagues (Chiodo et al., 1984) were the first to demonstrate that a group of dopamine neurons, antidromically stimulated from the prefrontal cortex, fire on average, at ~9.3 Hz - three times higher than the typically described average rate of nigrostriatal dopamine neurons ~3.1 Hz (their data). Recent *in vitro* studies have corroborated Chidodo’s finding, and thus by convention, it is now thought that the classic low firing rates are specific to the dorsal striatal and lateral shell accumbal projecting dopamine neurons, while the mesocorticolimbic dopamine subpopulations located in the medial VTA fire at much higher basal rates: 3 - 6 Hz vs 10 - 15 Hz (Lammel et al., 2008). Till date, these are the only studies that have examined the burst firing of dopamine neuron subpopulations *in vivo* and *in vitro* experiments.

In the phasic mode of firing, dopamine neurons fire a burst of sequence of action potentials, enabling a more efficient release of dopamine from their terminals as compared to regular pacemaking (Floresco et al., 2003). From *in vivo* rodent studies, it has been observed that dopamine neurons can naturally burst fire, the pattern and features of which are similar to bursts observed in awake behaving non-human primates (Grace and Bunney, 1984b; Schultz, 1986; Overton and Clark, 1997; Paladini and Roeper, 2014). In awake primates, bursts were observed to be time-locked to the presentation of free or conditioned reward, some in response to aversive stimuli, cue presentation, or locomotion (Schultz, 1986; Schultz et al., 1993; Schultz, 2007; Matsumoto and Hikosaka, 2009; Jin and Costa, 2010; Barter et al., 2015; Howe and Dombeck, 2016). At the cellular level, a burst of dopamine neurons results in a high amplitude and synaptic release of dopamine, thought to be necessary for relaying the necessary teaching
signal to downstream neurons in the target region (Paladini and Roeper, 2014; Grace, 2016). Mechanistically, that this could be achieved by changing the occupancy level of the low-affinity D1 and high-affinity D2 receptors on the striato-nigral medium spiny neurons, which would change their synaptic weights and signaling levels, and result in an update in the element of expectation in the circuit (Paladini and Roeper, 2014). Electrophysiologically, burst firing occurs as a series of successive action potentials with decreasing amplitude and increasing interspike intervals (Grace and Bunney, 1984b; Marinelli et al., 2006). In vivo, bursts are defined as displaying at least three spikes beginning with an interspike interval of 80 ms and ending with an ISI of 160 ms or longer (Grace et al., 1984b). Notably, the naturally occurring burst firing pattern is not a usual characteristic of dopamine neurons in vitro. This is generally considered to be due to the loss of the excitatory inputs present in vivo (Marinelli et al., 2006; Morikawa and Paladini, 2011). While the bursting pattern is much rarer in vitro, when it does occur, it has been observed more often in VTA dopamine neurons compared to the mesostriatal neurons (Korotkova et al., 2004). Similarly, with the use of current injections in in vitro midbrain slices, it is possible to elicit high frequency firing in dopamine neurons that is similar in pattern to the burst firing observed in vivo (Blythe et al., 2009). It is also possible with the use of pharmacological agents such as glutamate, to change a non-bursting dopamine neuron to burst fire in vitro, however under such conditions, the firing pattern does not appear to be the same (Korotkova et al., 2004). In vivo, bursting confers a tighter coefficient of variation, resulting in little variability, however the opposite appears to be the case with pharmacologically-induced bursts in slice preparations (Marinelli et al., 2006).

Lastly, it should be noted that from in vivo recordings, not all dopamine neurons are active when examined. At the behavioral level, during awake behaving rodent and non-human primate studies, pauses have been shown to be elicited in response to reward omission (Mirenowicz and Schultz, 1996) and in response to the presentation of an aversive stimulus (Schultz et al., 1993; Matsumoto and Hikosaka, 2009). At the cellular level, the pause is
associated with a decrease or halt in the activity of the dopamine neurons, which is also reflected in the amount of dopamine released. Interestingly, the nature of the pause response is heterogeneous among the dopamine neurons, as SNc dopamine neurons will display shorter latency pauses in response to aversive stimuli as compared to VTA dopamine neurons (Brown et al., 2009; Mileykovskiy and Morales, 2011). However, whether this difference in pauses exists among dopamine neuron subpopulations and can be seen at the cellular level remains unknown.

In terms of the electrophysiology recordings of the dopamine neurons in this state, it was found that about 1/3rd of dopamine neurons are silent, with particularly hyperpolarized membrane potentials (~ -65 to -70 mV), and could be induced into a state of bursting activity by the application of either glutamate or dopamine antagonists (Bunney et al., 1991). The burst spikes from the evoked dopamine neurons were found to be similar in pattern and form to spikes of naturally firing dopamine neurons. It is believed that these neurons are under more GABAergic afferent control and hence kept silent. These instances of pauses or periods of silence are usually studied in vitro by evoking GABAergic pauses through electrical stimulation of the afferents or GABA uncaging. It should be noted however that silent dopamine neurons encountered in vitro are not usually recorded from, as such, data from dopamine neurons in this state is sparse. There are also additional forms of pauses that are typically encountered in vitro, such as the pauses that follow bursts known as the post-burst pause, and dopamine inhibitory pauses. The mechanisms that regulate these pauses are believed to be distinct from the GABAergic induced pauses, and have remain largely unexplored among the dopamine neuron subpopulations. Similarly, pauses can be elicited from other synaptically induced sources via MGluR1 and adrenoreceptors.
1.6 IONIC CONDUCTANCES THAT UNDERLIE THE HETEROGENEITY IN THE EXCITABILITY OF MIDBRAIN DOPAMINE NEURON SUBPOPULATIONS.

1.6.1 PACEMAKING IN SNC vs VTA DOPAMINE NEURONS –

*In vitro*, the majority of the midbrain dopamine neurons fire tonically, also known as pacemaking, even though the innervations from most excitatory and inhibitory innervations have been cut. As such, intrinsic mechanisms are responsible for the pacemaking feature of dopamine neurons. Pacemaking in dopamine neurons is generally characterized by long duration, voltage-dependent and oscillatory action potentials that are mediated by calcium, sodium and potassium currents (Grace and Onn, 1989; Yung et al., 1991; Puopolo et al., 2007; Kimm et al., 2015). These action potentials may sometimes display an after-hyperpolarization that is calcium dependent (Wolfart et al., 2001). The voltage during the interspike interval is a slow depolarization that is shaped by subthreshold calcium, sodium and potassium conductances (Mercuri et al., 1994; Khaliq and Bean, 2008, 2010). When the action potentials of spontaneously firing SNc dopamine neurons are blocked by the selective sodium channel blocker, tetrodotoxin (TTX), a slow oscillatory potential (SOP) is unmasked (Nedergaard et al., 1993; Marinelli et al., 2006), which is absent in a fraction of VTA dopamine neurons. When comparing the pacemaking behavior of SNc and VTA dopamine neurons, they display differences in both their firing rates and coefficient of variation. SNc dopamine neurons tend to fire at lower rates with a tighter coefficient of variation as compared to VTA dopamine neurons (Neuhoff et al., 2002; Marinelli et al., 2006; Schiemann et al., 2012; Paladini and Roeper, 2014). As such, some studies have examined and compared the ionic currents, both spike-triggered and subthreshold, that underlie pacemaking but in the context of the two regional locations of the midbrain dopamine neurons – the SNc vs VTA. These studies have demonstrated that gross differences can be seen in the ionic conductances that underlie the firing heterogeneity between the SNc and the VTA. While most studies have examined the somatodendritic voltage-gated currents of the SNc neurons, very few have examined the somatic currents in VTA DA neurons,
and almost no studies have focused examining these currents in the context of their subpopulations. As such, much of the data on ionic currents in the SNc and VTA includes variability that could be further broken down by projection patterns of the dopamine neurons. The heterogeneity of the ionic currents among the dopamine neuron subpopulations and how they contribute to the diversity in the excitability properties of these subpopulations remains an open question.

The slow oscillatory potentials in dopamine neurons are mediated by somatodendritically located low threshold L-type calcium conductances (Wilson and Callaway, 2000; Chan et al., 2007; Puopolo et al., 2007; Guzman et al., 2009; Hage and Khaliq, 2015). These currents are activated and inactivated in the subthreshold voltage range, making them ideal in shaping pacemaking in the dopamine neurons. Recent work has demonstrated that L-type calcium currents display large amplitudes and inactivate on a slower time scale in the SNc dopamine neurons, as compared to VTA dopamine neurons (Puopolo et al., 2007; Philippart et al., 2016). As such, this slow oscillatory potential is not observed in certain VTA dopamine neurons. In SNc dopamine neurons, Cav1.3 has been identified as the L-type channel that mediates this current (Chan et al., 2007). In terms of pharmacology, they are selectively blocked by the dihydropyridine compounds like nifedipine, nisoldipine and isradipine, many of which are currently used in the mainstream consumer market as antihypertension pharmaceutical drugs. Interestingly, one hypothesis* in the field is that the same calcium channels that underlie the pacemaking of dopamine neurons makes them particularly vulnerable to mitochondrial oxidative stress, and hence the selective death of these neurons in Parkinson’s disease (Chan et al., 2007; Guzman et al., 2009). This is believed to be one possible reason as to why SNc dopamine neurons die first in Parkinson’s disease as compared to VTA dopamine neurons. However, a clear characterization and comparison of the L-type calcium currents is yet to be

* commonly termed the calcium hypothesis.
done among the dopamine neuron subpopulations.

The characteristic slow AHP in dopamine neurons is produced by the calcium-activated small conductance potassium currents (SK), which are potassium conductances that are activated by increases in cytoplasmic calcium (Grace and Bunney, 1984a; Mercuri et al., 1994; Wolfart et al., 2001). SK channels are thought to be inextricably linked to calcium channels as they have little voltage dependence of their own and instead, derive it from calcium channels (Hille, 2001). Accordingly, both low threshold L-type (Bowden et al., 2001) and T-type channels (Wolfart and Roeper, 2002) have been shown to be coupled with SK channels. However, evidence also exists that SK channels can also be activated by calcium from internal calcium stores opened due to the activation of metabotropic MGluR1 receptors (Morikawa et al., 2003). During spontaneous firing, SK currents are present at the end of an action potential and beginning of the interspike interval, thus modulating the precision of spike firing, as the block of SK results in irregular and higher rates of firing - regarded as a type of burst (Wolfart et al., 2001). As such, this difference in the AHP current is believed to be a contributing reason as to why precise firing is regularly seen in SNc as compared to VTA dopamine neurons (Wolfart et al., 2001). RT-PCR data has shown the abundance of SK3 mRNA in SNc, with much less in VTA dopamine neurons overall (Wolfart et al., 2001; Dufour et al., 2014). However, neither the AHP nor SK currents have been examined among the dopamine neuron subpopulations. The bee venom derived toxin, apamin, is pharmacologically used to block these channels.

Dopamine neurons display a distinct sag in response to hyperpolarization, demonstrated to be mediated by currents through the hyperpolarization-activated cyclic nucleotide (HCN) channel (Neuhoff et al., 2002). The sag itself was once dubbed to be due to a ‘funny current’ or called I_f in the cardiac myocytes cells it was first identified in. It was called so because it activates at very hyperpolarized thresholds, pulling the membrane to depolarized potentials, and portraying a ‘sag’ in the process (Luthi and McCormick, 1998). The I_h current is voltage dependent, and responsive to the cell’s level of cyclic AMP (cAMP) (Hille, 2001; Amendola et
al., 2012). Before the development of transgenic mice that express a fluorescent (GFP or Td tomato) tagged to a robust maker (tyrosine hydroxylase (TH) or dopamine transporter (DAT)) of the dopamine neurons, the sag had been routinely used as an electrophysiological marker that was thought to be reliable in the identification of all midbrain dopamine neurons (Ungless and Grace, 2012). However, with the use of these mice to identify dopamine neurons, it has been discovered that \( I_{h} \) is not a reliable marker of dopamine neurons, as not all dopamine neurons, particularly among the VTA dopamine neurons portray the sag (Lammel et al., 2008; Margolis et al., 2008). Immunostaining experiments have demonstrated the somatodendritic presence of HCN2 and HCN4 channels in the SNc dopamine neurons, which is consistent with their role in limiting the temporal summation of innervating excitatory inputs in SNc as compared to VTA dopamine neurons (Dufour et al., 2014; Masi et al., 2015). Interestingly, even among the SNc dopamine neurons, \( I_{h} \) has been shown to be less ubiquitous than previously thought as some SNc DA neurons exhibit a slight reduction in their firing frequency, while it has no effect in a larger portion of SNc dopamine neurons (Neuhoff et al., 2002; Puopolo et al., 2007). As such, as the blockade of \( I_{h} \) never halts firing, it is thought to be a contributive rather than dominant current in the pacemaking of SNc dopamine neurons. Among the SNc dopamine neurons, the amplitude of the \( I_{h} \) sag has been found to be heterogeneous, in one instance based on their projection target (Lerner et al., 2015), and in another can be associated with the presence or absence of a molecular marker like the calcium binding protein, calbindin (Neuhoff et al., 2002; Evans et al., 2017). Pharmacologically, HCN channels can be blocked with specificity using the compound ZD7288 or Ivabradine.

The slow pacemaker frequency of dopamine neurons is enabled in part by the presence of the subthreshold A-type potassium current. It is a transiently activating and inactivating potassium current that prevents depolarization during the interspike interval of the SNc dopamine neurons, thereby contributing to setting the firing frequency (Liss et al., 2001; Khaliq and Bean, 2008). It is also the dominant outward current in the interspike interval of dopamine
neurons (Khaliq and Bean, 2008). Evidence also shows that A-type potassium currents regulate the recovery from hyperpolarization of the membrane potential (rebound delay) (Amendola et al., 2012). In the SNc dopamine neurons, A-type potassium currents are thought to be produced by the K\textsubscript{v}4.3 channels in dopamine neurons and distributed somatodendritically (Liss et al., 2001; Gentet and Williams, 2007; Dufour et al., 2014). Despite the difference in the range of firing frequencies among the dopamine neurons of the SNc and VTA, previous literature has not demonstrated a difference in density in A-type potassium channels. These channels are also yet to be examined in the context of the dopamine neuron subpopulations. Pharmacologically, these channels can be selectively blocked by the scorpion derived toxin, AmmTX3 (Vacher et al., 2002).

Dopamine neurons possess both transient and persistent sodium currents that enable action potentials to fire, although, the sodium channel density among the VTA and SNc dopamine neurons are yet to be compared. However, the sodium channel density is reported to be lower, and have slower deactivation kinetics in the SNc dopamine neurons compared to the fast-spiking GABAergic neurons of the substantia nigra reticulata (Seutin and Engel, 2010; Ding et al., 2011a). Interestingly, when examining subthreshold sodium currents during pacemaking, although they play a contributive role, they are of a smaller amplitude compared to the subthreshold calcium currents in SNc dopamine neurons (Puopolo et al., 2007). On the other hand, when subthreshold sodium and calcium currents are compared in the medially located VTA dopamine neurons that form the mesocorticolimbic pathway, the sodium currents recorded are larger in amplitude as compared to the calcium currents (Khaliq and Bean, 2010). Additionally, a non-selective cationic current believed to be mediated by Na\textsuperscript{+} currents and acts in driving pacemaking, was demonstrated to be present among the VTA dopamine neurons as compared to the SNc dopamine neurons (Khaliq and Bean, 2010). However, the identity of the channel that mediates this current remains unknown. The voltage-dependent sodium channels that conduct the sodium currents in dopamine neurons are believed to be via the ubiquitously
expressed neuronal sodium channels Na\textsubscript{V}1.1 and Na\textsubscript{V}1.2. Interestingly, there is also evidence of the sodium channel associated with resurgent sodium currents, Na\textsubscript{V}1.6 documented among the SNc dopamine neurons, although the resurgent currents recorded were of diminutive amplitudes compared to that in the SNr GABA neurons (Ding et al., 2011a). Knowledge on the different sodium channels and how their densities differ among the dopamine neuron subpopulations remains lacking.

The high threshold potassium currents that enable action potential repolarization have been the least studied currents among the dopamine neurons. Among the SNc dopamine neurons, K\textsubscript{V}2 and BK currents have been shown to play redundant and compensatory roles in repolarizing the membrane during an action potential, and do not significantly impact the interspike interval or pacemaking frequency (Kimm et al., 2015). However, BK and K\textsubscript{V}2 currents have not been studied among the VTA dopamine neurons, and it remains unknown how they compare to the currents in SNc dopamine neurons. K\textsubscript{V}3 currents have been shown to be present via electrophysiology, immunostaining and TRAP-mRNA data among the SNc and VTA dopamine neurons (Ding et al., 2011b; Dufour et al., 2014; Brichta et al., 2015). In response to large step depolarizations, the amplitude of K\textsubscript{V}3 currents is comparatively lower among SNc dopamine neurons as compared to SNr GABAergic neurons (Ding et al., 2011b). Among the dopamine neurons however, there are no comparative electrophysiological studies that have been carried out to examine the K\textsubscript{V}3 currents. However, TRAP-mRNA data has demonstrated a higher fold presence of K\textsubscript{V}3 mRNAs in VTA dopamine neurons as compared to SNc dopamine neurons (Brichta et al., 2015). Similarly, the contribution of K\textsubscript{V}3 currents to action potential repolarization among the dopamine neuron subpopulations remains to be explored.

1.6.2. BURST FIRING OR HIGH FREQUENCY FIRING IN SNc vs VTA DOPAMINE NEURONS

In vivo, dopamine neurons have been observed to burst naturally as seen in rodent recordings (Grace and Bunney, 1984b), where synaptic inputs from innervating brain regions
are still intact. As such, burst firing is rarely observed naturally \textit{in vitro}, unless induced by the use of pharmacological agents like glutamate or the electrical stimulation of afferent fibers. As briefly mentioned in \textit{Section III}, dopamine neurons receive varied inputs, some of which are excitatory and thus, provide the drive for bursting. Many of the inputs that innervate both the SNc and VTA dopamine neurons have been demonstrated to elicit burst firing, most prominently from the pedunculopontine nucleus, lateral dorsal tegmentum and subthalamic inputs - regions that heavily innervate the dopamine neurons (see \textit{Section III} and (Mercuri et al., 1996; Tong et al., 1996b, a; Watabe-Uchida et al., 2012)). The use of NMDA (N-methyl-D-aspartate) antagonists, and in some studies, AMPA (\(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), decreases the number of bursting neurons, although there is no actual consensus on if the latter is necessary (Mercuri et al., 1996; Korotkova et al., 2004; Blythe et al., 2009). Calcium influx through NMDA receptors been shown to be necessary for bursts, as \textit{in vivo} bursting is abolished in the presence of calcium chelators (Grace and Bunney, 1984b), and knockout of NMDA in dopamine neurons abolishes bursts without affecting their spontaneous firing activity (Zweifel et al., 2009).

The ionic conductances that regulate the burst mode of firing are thought to be different from those that regulate pacemaking. Studies on bursting in dopamine neurons have focused on the identifiable dopamine neurons using the popularly used electrophysiological markers. Therefore, the information on dopaminergic neuron bursting is most likely from SNc and lateral VTA dopamine neurons. Aside from afferent fiber stimulation, \textit{in vitro} bursting can be observed by the application of apamin, the SK channel blocker, and is thought to be mediated by L-type calcium channels (Ping and Shepard, 1996). As mentioned above, SK and L-type calcium conductances are negligible in some VTA dopamine neurons; therefore, induced bursting may occur via other intrinsic conductances in these neurons. K-ATP channel co-activation with NMDA receptors has also been demonstrated to contribute to burst firing among the SNc dopamine neurons (Schiemann et al., 2012). Additionally, recent work has demonstrated a post-
inhibitory (either by dopamine or current injections) burst that is mediated by T-type calcium currents and present in calbindin-negative SNc dopamine neurons (Evans et al., 2017). However, the expression of these ion channels in the VTA dopamine neurons, particularly in the context of their subpopulations also remain unknown.

The extent to which a dopamine neurons can burst fire is limited by the inactivation of the sodium channels, which is typically referred to as depolarization block. As some dopamine neurons can fire at higher frequencies compared to others, there is a possibility that there exists a difference in the inactivation properties of sodium channels among the dopamine neuron subpopulations, which is yet to be explored (Grace and Bunney, 1986). One published report has demonstrated that dopamine neurons can be induced to burst in a similar pattern as observed in vivo with sufficient depolarizing current injections, although this is not consistently reproducible (Blythe et al., 2009; Morikawa and Paladini, 2011). Lastly, the high threshold potassium currents produced by Kv2 and BK channels, have recently been demonstrated to play opposing roles in shaping burst firing among the SNc dopamine neurons (Kimm et al., 2015). In dissociated SNc dopamine neurons, it has been demonstrated that Kv2 currents limit high frequency firing, while BK currents enhance it, most likely due to differences in their activation and deactivation properties.

While it is hard to recapitulate in vivo burst firing, most in vitro studies still use current injections and rely on frequency-intensity curves to carry out an approximate study of burst phenomena. High frequency firing obtained in vitro in one reported instance has been compared to display a similar pattern as seen in burst firing obtained in vivo (Blythe et al., 2009). As such, frequency-intensity curves obtained from in vitro experiments remain a fair way to uncover the intrinsic mechanisms of burst firing that can be engaged in vivo. Previous reports examining dopamine neurons in intact in vitro midbrain slices have demonstrated a diversity of high frequency firing rates among the dopamine neuron subpopulations (Lammel et al., 2008; Margolis et al., 2008). However, till date, the intrinsic ionic mechanisms that contribute to the
differences in high frequency firing among these subpopulations remain elusive.

1.6.3 PAUSES IN SNC VS VTA DOPAMINE NEURONS

Dopamine neurons pause their activity when either reward is omitted or, in some neurons, when an aversive stimulus is presented (Mirenowicz and Schultz, 1996; Matsumoto and Hikosaka, 2009). Using in vitro preparations, researchers have attempted to understand the postsynaptic currents that facilitate the synaptic initiation of pauses. However, one key challenge to answering this question is that naturally occurring pauses are observed more frequently when recording dopamine neurons from in vivo preparations, compared to in vitro preparations. As such, naturally occurring in vivo pauses are often mimicked by either stimulating GABAergic inputs to directly inhibit dopamine neurons or by activating D2 autoreceptors. Another common way that pauses are observed in vitro is their co-occurrence with bursts. When bursts are synaptically elicited, they are typically followed by a pause before the neuron returns to spontaneous activity (Paladini and Roeper, 2014). These two types of pauses - those that occur in the absence of burst phenomena, and the post-burst pause are thought to be regulated by different inhibitory routes and ionic mechanisms.

Pauses can be elicited from the direct activation of GABAergic afferents that inhibit the activity of dopamine neurons. Dopamine neurons receive several inhibitory afferents, however, the SNC is under far more inhibitory control than the VTA (Tepper and Lee, 2007). Both GABA_A and GABA_B receptors are activated by inhibitory afferents onto the dopamine neurons, although the ionic mechanisms that underlie them are different (Paladini et al., 1999; Paladini and Tepper, 1999). Synchronous activation of GABA_ARs on dopamine neurons is believed to be needed for long durations of silence to be elicited in the dopamine neurons, particularly given the depolarized reversal potential of chloride in the dopamine neurons (Gulacsi et al., 2003; Lobb et al., 2011). On the other hand, GABA_B inputs have been known to be controversial in the

* This type of pause is also referred to as the post-burst pause

25
dopamine neurons, displaying bidirectional activity depending on the duration of activation. Activation of GABA\(_B\) receptors has been shown to decrease burst firing, as these receptors are coupled to GIRK channels in the dopamine neurons (Engberg et al., 1993; Labouebe et al., 2007). However, using GABA uncaging in dopamine neurons, it was shown that the transient activation of GABA\(_B\) receptors leads to the inhibition of SK currents in SNc dopamine neurons and as such, increased burst firing in these neurons (Estep et al., 2016). From this work, one proposed idea is that a GABA\(_A\) and GABA\(_B\) pattern of transient activation can elicit a burst-pause excitability pattern in SNc dopamine neurons. However, more recent work has shown that accumbal inputs preferentially synapse onto GABA\(_B\) receptors on the dopamine neurons and GABA\(_A\) receptors on the VTA GABAergic neurons (Edwards et al., 2017). This raises the possibility that different inhibitory nuclei can exert control over the dopamine neurons via separate GABA receptors, and illuminates a layer of regulatory control at the receptor level that the dopamine neurons can have over incoming inputs to control their level of excitability.

Dopamine neurons can also electrically silence their own activity by inducing a pause via their dopamine receptors (DAR). In brief, dopamine receptors, which include D1 – D5, are members of the seven-transmembrane superfamily of G-protein-coupled receptors (GPCRs) (Ford, 2014). Dopamine neurons express D2 receptors (D2R) which together with D2, D3 and D4 form the D2-like receptors and are inhibitory via G\(_i/o\) signaling. Dopamine neurons express their autoreceptors somatodendritically and at their terminals. As such, they can directly regulate their firing activity via activation of potassium currents and indirectly by affecting Tyrosine Hydroxylase (TH) and DAT expression thus affecting extracellular dopamine levels (Ford, 2014). Somatodendritically located D2 autoreceptors are bound by extracellular dopamine, which activates downstream signaling cascades and results in the opening of G-protein activated potassium (GIRK) channels (Lacey et al., 1987, 1988; Beckstead et al., 2004). GIRK channels mediate a hyperpolarizing K\(^+\) current that temporarily silences the neuron’s firing. This was another commonly used criterion for dopamine neuron identification in early
electrophysiological studies. However, Chiodo and his colleagues (Chiodo et al., 1984) were the first to demonstrate that dopamine neurons that project to the prefrontal cortex do not change their firing rate in response to dopamine, meaning that they do not possess D2 autoreceptors. Recent work has confirmed this fact, and has further shown that the prefrontal cortex projecting dopamine neurons express low mRNA levels of the dopamine transporter (DAT), vesicular monoamine transporter 2 (VMAT2), and GIRK mRNA (Lammel et al., 2008). Similar work in rats demonstrated that the amygdala projecting dopamine neurons lack dopamine autoinhibition (Margolis et al., 2008). As such, although dopamine neurons can possess a powerful autoregulatory mechanism of pausing their firing activity, this itself is heterogeneous, as it is lacking in some dopamine neuron subpopulations. The observation of the lack of D2 autoreceptors among the prefrontal projecting and other mesocorticolimbic dopamine neuron subpopulations in the rodent VTA, together with the increased firing rates observed in these same neurons has fueled the idea that the level of spontaneous activity of dopamine neurons is determined by the basal level of autoreceptor activation (White and Wang, 1984b). As such autoreceptor activation would be also expected to limit burst activity in the dopamine neurons. However, while this idea has been widely accepted as a contributing mechanism to the differences in excitability among the subpopulations of the dopamine neurons, it remains to be tested.

Bursts can also be induced by the activation of mGluRs (Prisco et al., 2002), and are typically accompanied by a long-lasting hyperpolarization or pause (Morikawa et al., 2000; Morikawa et al., 2003). Dopamine neurons express functional mGluR1 receptors which are Gq-coupled, and activate phospholipase C (PLC) which leads to the hydrolysis of phosphotidylinositol-4,5-diphosphate (PIP2) into inositoltriphosphate (IP3) and diacylglycerol (DAG). The increase in IP3 leads to the release of calcium from intracellular calcium stores, which in turn activates SK channels (Morikawa et al., 2000). The SK currents in turn hyperpolarizes the membrane potential eliciting a pause that can last up to seconds. Similar
observations have been noted with activation of the adreno-receptors on dopamine neurons (Paladini and Williams, 2004). Thus, the sequential activation of NMDA and mGluR1 or adreno-receptors can drive the burst-pause sequence. It should be noted that these observations are with respect to dopamine neurons that possess SK channels. Alternative ionic mechanisms that underlie the burst-pause maybe be present in non-SK expressing dopamine neurons, and remain to be elucidated.

Most of the information on pauses in dopamine neurons has been garnered from experiments with SNc dopamine neurons with very few in VTA dopamine neurons, much less dopamine neuron subpopulations. Thus, the synaptic and intrinsic mechanisms underlying pauses among the dopamine neurons remain an important and open question.

1.7 SUMMARY

Midbrain dopamine neurons regulate a variety of essential behaviors including reward signaling, aversion, reinforcement, locomotion and motivation among others. They have been implicated in many different pathophysiological conditions including Parkinson’s disease, depression, ADHD and schizophrenia. Dopamine neurons are believed to support these broad range of behaviors by sending their axonal projections to different brain regions, thus participating in disparate brain circuits. As such, understanding dopamine neurons in the context of the brain circuits they participate in means studying them in the context of their subpopulations. However, despite the highlighted heterogeneity of midbrain dopamine neuron subpopulations in the behaviors they support, circuit architecture and gross excitability properties, the cellular bases of differential signaling in the different dopamine neuron subpopulations remain unknown. The versatile involvement of these neurons in far-reaching and fundamental neural circuits underscores the need to understand the contributing ionic mechanisms that underlie their functionality at the cellular level. One basic question that my dissertation strives to answer is this: how are the intrinsic currents in different dopamine neuron
subpopulations expressed to produce the neural correlates that they display in response to environmental cues? As such, in this dissertation, I hope to answer this overarching question by examining excitability states in dopamine neurons, more specifically, examining intrinsically produced pauses and high frequency firing. I employ the use of retrograde labeling, patch-clamp electrophysiology and, through a collaboration, computational modeling, to explore the ionic mechanisms that contribute to the differences observed in pauses and high frequency firing among the dopamine neuron subpopulations. In chapter 2, I explore the ionic mechanisms that underlie the well described pause in dopamine neurons, which has been demonstrated to be a correlate of aversive signaling and reward omission in dopamine neurons. I describe the novel differential contribution of the subthreshold A-type potassium currents and H-currents to eliciting different temporal pauses in the accumbal and striatal projecting dopamine neuron subpopulations. In chapter 3, I re-examine the phenomenon of differential firing frequencies among the dopamine neuron subpopulations, with an attempt at uncovering the ionic mechanisms that contribute to such differences. I find that SK currents do limit high frequency firing in accumbal and striatal projecting dopamine neurons as opposed to prefrontal projecting dopamine neurons. I also identify an interesting build-up of K\textsubscript{V}2 currents during high frequency firing that is slower in SNc as compared to mesocortical and unlabeled VTA dopamine neurons.

In conclusion, this body of work adds to the growing evidence on the diversity of dopamine neuron subpopulations and the continual need to uncover the mechanisms that contribute to said diversity.
Figure 1-1: A topographical illustration of the midbrain dopamine neurons in the SNc and the VTA.

Top, A coronal section depicting the spatial projection pattern of the dopamine neuron axons to the caudate-putamen, ventral striatum and olfactory tubercle. Bottom (a-f), Dorsal to Ventral slices of zoomed in coronal sections of the midbrain dopamine neurons that shows a differential distribution of the neurons. This figure was adapted from (Ikemoto, 2007).
Figure 1-2 The somatodendritic activation of D2 autoreceptors elicits an inhibitory post synaptic current that hyperpolarizes dopamine neurons and results in their quiescence (pause).

Left, A schematic diagram depicting the efflux of dopamine, activation of D2 receptors and GIRK channels in dopamine neurons which results in the efflux of potassium ions. Right, Recording of a D2-IPSC evoked from a train of extracellular stimuli which led to the local release of dopamine. Note the D2-IPSC causes a pause in the firing of a VTA dopamine neuron. This figure was adapted from (Ford, 2014).
CHAPTER 2

ENHANCED SENSITIVITY TO HYPERPOLARIZING INHIBITION IN MESOACCUMBAL RELATIVE TO NIGROSTRIATAL DOPAMINE NEURON SUBPOPULATIONS.

Rahilla A. Tarfa¹,², Rebekah C. Evans² and Zayd M. Khaliq²

¹Department of Neuroscience, Brown University, Providence, RI USA
²Cellular Neurophysiology Unit, National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH), Bethesda, MD, USA

Author Contributions: R.A.T. and Z.M.K. designed the experiments. R.A.T. conducted the experiments and analyzed the data. R.C.E. generated the computational model and performed simulations. The manuscript was written and edited by R.A.T. and Z.M.K.

This Chapter is adapted from the manuscript published in the Journal of Neuroscience: DOI: https://doi.org/10.1523/JNEUROSCI.2969-16.2017
2.1 ABSTRACT

Midbrain dopamine neurons recorded *in vivo* pause their firing in response to reward omission and aversive stimuli. While the initiation of pauses typically involves synaptic or modulatory input, intrinsic membrane properties may also enhance or limit hyperpolarization, raising the question of how intrinsic conductances shape pauses in dopamine neurons. Using retrograde labeling and electrophysiological techniques combined with computational modeling, we examined the intrinsic conductances that shape pauses evoked by current injections and synaptic stimulation in subpopulations of dopamine neurons grouped according to their axonal projections to the nucleus accumbens or dorsal striatum in mice. Testing across a range of conditions and pulse durations, we found that mesoaccumbal and nigrostriatal neurons differ substantially in their rebound properties with mesoaccumbal neurons displaying significantly longer delays to spiking following hyperpolarization. The underlying mechanism involves an inactivating potassium ($I_A$) current with decay time constants of up to 225 ms, and small-amplitude hyperpolarization-activated currents ($I_H$), characteristics that were most often observed in mesoaccumbal neurons. Pharmacological block of $I_A$ completely abolished rebound delays and, importantly, shortened synaptically evoked inhibitory pauses, thereby demonstrating the involvement of A-type potassium channels in prolonging pauses evoked by GABAergic inhibition. Therefore, these results show that mesoaccumbal and nigrostriatal neurons display differential responses to hyperpolarizing inhibitory stimuli that favors a higher sensitivity to inhibition in mesoaccumbal neurons. These findings may explain, in part, observations from *in vivo* experiments that ventral tegmental area neurons tend to exhibit longer aversive pauses relative to SNc neurons.
2.2 INTRODUCTION

Midbrain dopamine neurons contribute to a range of behaviors including reward, aversion, and movement. Early studies classified dopamine neurons based on their anatomical location within either the ventral tegmental area (VTA) or the substantia nigra (SNc) (Dahlstroem and Fuxe, 1964). However, individual dopamine neurons innervate only a single brain nucleus and thus may be more effectively classified according to their axonal projections (Fallon and Moore, 1978; Oades and Halliday, 1987; Bjorklund and Dunnett, 2007; Aransay et al., 2015). In particular, a subset of VTA dopamine neurons project to the nucleus accumbens (mesoaccumbal), while most SNc neurons project primarily to the dorsal striatum (nigrostriatal). Determining whether mesoaccumbal and nigrostriatal neurons form functionally distinct subpopulations is an important step in understanding how dopamine-dependent signaling contributes to reward and motor circuits. Dopamine neurons recorded in vivo pause their firing following reward omission or in response to aversive stimuli (Schultz et al., 1997; Ungless et al., 2004; Fiorillo et al., 2013a,b). Interestingly, the duration of pauses varies with cell location within the midbrain. For example, VTA neurons reliably pause their firing in response to aversive stimuli (Mileykovskiy and Morales, 2011; Wang and Tsien, 2011), while SNc neurons respond more variably with either decreasing or increasing excitability (Matsumoto and Hikosaka, 2009; Lerner et al., 2015) or do not respond at all (Brown et al., 2009). One study compared pauses in vivo following spontaneously generated bursts in dopamine neurons, and found that mesoaccumbal neurons exhibited significantly longer post burst pauses than nigrostriatal neurons (Clark and Chiodo, 1988). Although the observed heterogeneity in pauses among dopamine neurons subpopulations likely involves differences in synaptic inputs, whether differences in intrinsic membrane conductances contribute has yet to be fully determined. Cellular-level studies examining membrane responses to prolonged hyperpolarizations have provided important insight into the ionic conductances that shape the rebound properties of substantia nigra neurons (Neuhoff et al., 2002; Amendola et al., 2012). These studies
demonstrate that post inhibitory rebound delays rely on the interplay of transient outward potassium (I_A) currents along with hyperpolarization-activated cation (I_h) currents. However, whether rebound delays are predictive of responses to inhibitory stimuli that occur at the subthreshold voltages achieved during natural spontaneous activity is not well understood. Furthermore, experiments testing the intrinsic conductances that shape synaptically evoked inhibitory (i.e., GABAergic) pauses in dopamine neurons have not yet been performed, and whether these underlying ionic conductances differ between mesoaccumbal and nigrostriatal dopamine neuron subpopulations is not well understood. We used retrograde labeling and electrophysiology in combination with computational modeling to compare rebound properties, post-burst pauses and synaptically evoked inhibitory pauses in mesoaccumbal and nigrostriatal dopamine neuron subpopulations. We found that mesoaccumbal neurons exhibit substantially longer rebound delays than nigrostriatal neurons in response to hyperpolarizing current injections covering a range of amplitudes and durations. Recording the underlying ionic currents in voltage-clamp mode, we found that the higher sensitivity of mesoaccumbal neurons relies on recruitment of A-type potassium currents that display slow inactivation kinetics. By contrast, nigrostriatal neurons expressed I_A currents that displayed faster inactivation kinetics and larger amplitude I_h currents. Computational modeling demonstrated that the slow decay of I_A alone slows rebound responses to hyperpolarizing inhibition, even in the presence of sizeable I_h and T-type calcium currents. Last, we tested the pharmacological block of I_A on pauses evoked by GABAergic synaptic inputs and found that I_A enhances GABA mediated pauses in dopamine neurons. Together, these experiments demonstrate that mesoaccumbal and nigrostriatal neurons display differential responses to hyperpolarizing inhibition. Furthermore, a unique combination of ionic conductances in mesoaccumbal neurons prolong pauses in firing and may consequently play an important role in signaling of aversive events. Given recent findings that dopamine neuron subpopulations receive largely overlapping synaptic inputs (Beier et al., 2015; Lerner et al., 2015; Menegas et al., 2015), these results suggest that heterogeneity in intrinsic
and integrative properties are equally important contributors to functional diversity among dopamine neuron subpopulations.
2.3 METHODS

**Animal Husbandry:** Experiments were performed on transgenic male and female mice (postnatal day 14 to 23) in which the expression of the green fluorescent protein (GFP) is driven by the promotor for tyrosine hydroxylase (TH-GFP mice) (Matsushita et al., 2002). All mice were maintained according to the guidelines set by the Animal Care and Use Committee for the National Institute of Neurological Disorders and Stroke and the National Institutes of Health.

**Stereotaxic Brain Injections:** A Stoelting stereotaxic instrument for small animals was used to microinject the retrograde labeler cholera toxin subunit B (CTB) conjugated to Alexa-555 (CTB-AF555) into the left and right hemispheres of TH-GFP mice at postnatal days 15 to 18. Mice were anesthetized using 1.5% isoflurane. Each scalp was shaved, cleaned with betadine and saline, injected with lidocaine (1%), and incised. PBS was used to keep the skull moist throughout surgery. The coordinates which correspond to bregma, lateral and ventral respectively were as follows: ±1.6, ±1.5, -4.6 for nucleus accumbens and ± 1.6, ± 1.4, - 2.5 for the dorsal striatum. CTB-AF555 (0.8 µl) was injected using a Hamilton microsyringe. After surgery, the scalp wound was closed using vet bond glue and the mouse was placed in an aerated cage to recover before being placed back in cage with parents and littermates. Mice were used for electrophysiology after a minimum of 3 days.

**Slice preparation:** Coronal midbrain slices of 300 m thickness were prepared from TH-GFP mice using a Microslicer DTK-Zero1. Slices were cut in an ice-cold glycerol-based slicing solution containing the following (in mM): 250 glycerol, 2.5 KCl, 2 MgCl₂, 2 CaCl₂, 1.2 NaH₂PO₄, 10 HEPES, 21 NaHCO₃, and 5 glucose. Slices were incubated in a warm 33°C bath containing recording solution simultaneously bubbled with 95% O₂/5% CO₂ for 30 min and incubated at room temperature for another 30 min. Recording solution contained the following (in mM): 125 NaCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 3.5 KCl, 10 glucose, 5 HEPES, 1 MgCl₂, and 2 CaCl₂.

**Patch-clamp electrophysiology:** Midbrain slices containing the VTA and SNc were placed in a heated recording chamber that was continuously perfused with heated ACSF with temperatures...
ranging from 31 to 33°C. Candidate neurons were first visualized and located using an upright Olympus BX50WI microscope connected to a Hamamatsu CCD camera. GFP-positive green neurons were dopamine neurons and CTB-AF555 positive red neurons were identified as retrogradely labeled subpopulations that innervate either nucleus accumbens or dorsal striatum. Neurons were recorded only if they were positive for both red and green fluorescence, indicating that they were dopamine neurons that projected to the either the nucleus accumbens or dorsal striatum.

Neurons were recorded using borosilicate recording electrodes (VWR International) pulled using a flaming/brown micropipette puller (Sutter Instrument). Pipettes were filled with an internal recording solution that contained the following (in mM): 135 KMeSO$_3$, 10 NaCl, 10 HEPES, 2 MgCl$_2$, 0.5 EGTA, and 0.1 CaCl$_2$. Electrodes were wrapped with Parafilm to reduce electrode capacitance. Brain slices were placed in a recording chamber that was constantly perfused with oxygenated recording solution. Slices were visualized with an upright microscope (Olympus) via an IR-DIC prism with 4and 60objectives. All data were collected using pClamp10 software (Molecular Devices), recorded on a Multiclamp 700B amplifier, and digitized on a Digidata 700B. Data were filtered at 10 kHz and sampled at 20 kHz. Series resistance and whole-cell capacitance were compensated using the whole-cell and R$_s$ compensation features and R$_s$ monitored frequently. Cells with uncompensated series resistances of 16 MΩ were immediately discarded. All experiments were performed at 31-33°C.

**Spontaneous and evoked firing.** To maximize the number of recordings displaying healthy firing activity, spontaneous firing was first monitored for a period in a cell-attached configuration. The membrane was then ruptured while recording in current-clamp mode, which allowed for easy identification of firing rate changes due to damage during breakthrough. Initial spontaneous firing activity was obtained for 1–2 min, during which the health of the neuron was further evaluated. If the spontaneous firing rate differed dramatically from the firing rate during the cell-attached configuration, the cell was discarded. Bridge balance was often corrected using the
automatic function of the Multiclamp amplifier. Frequency-intensity curves were obtained for each neuron by providing a series of depolarizing current injections (40 pA steps, 1 s duration) during their spontaneous activity. The maximal firing rate taken as the rate immediately preceding entry into depolarization block.

**Voltage-clamp recordings.** A-type potassium currents (I<sub>A</sub>) were isolated pharmacologically using extracellular recording solutions that included 50mM (tetraethylammonium chloride (TEA-Cl) and 1µM TTX. In some instances, 30 µM nifedipine, 20 µM ZD9866, 100 µM 4-aminopyridine, and 3 µM cesium chloride were present. A-type potassium currents exhibiting both the slow and fast inactivation kinetics were blocked in the presence of either 0.5 or 1 µM AmmTX3 (a specific blocker for Kv4 channels). The time constant of inactivation was measured in currents evoked by steps from 90 to 40 mV. The voltage dependence of inactivation was tested using a pulse to 40mV (250 ms) preceded by family of prepulse voltage steps (1 s) ranging from 120 to 40 mV.

**Histology and confocal imaging:** Slices with retrogradely labeled dopamine neurons were placed in 4% paraformaldehyde for at least 24 h and rinsed three times with PBS before being mounted onto glass slides with mounting medium (Vectashield). In Figure 1, slices were imaged using a Leica microscope with an inverted 10X objective. In Figure 2, neurons were imaged using a laser scanning confocal LSM 510 microscope using an inverted 10X objective for images of the whole tissue depicting injection site and 63 for the individual neuron (Fig. 2). Images were processed using ImageJ (NIH).

**Data analysis and statistics:** All electrophysiological traces were analyzed using Igor (Wavemetrics). To quantify voltage dependence of inactivation, current amplitudes resulting from a test pulse to 40 mV were plotted against prepulse voltages. Values were then fit with the following Boltzmann function:

\[
f(x) = I_{\text{max}} \left[ \frac{1}{1 + \exp \left( \frac{V - V_h}{k} \right)} \right] + I_{\text{min}}
\]
$I_{\text{min}}$, where $I_{\text{max}}$ equals maximal current, $V_h$ equals the voltage of half-inactivation, $k$ equals the slope factor, and $I_{\text{min}}$ equals the minimal current. Recovery from inactivation was obtained by plotting the ratio of the amplitudes of the second pulse (test) to the first pulse to the time interval. The curve was fit to a rising exponential function to obtain the time constant of recovery from inactivation. To calculate the recovery from inactivation, the fraction of recovery was calculated as the ratio of the recovered current amplitude to the amplitude of the test pulse. The fraction of recovery was then plotted against the corresponding duration of the recovery step between pulses and fit with an exponential to obtain the recovery time constant. The junction potential of our methanesulfonate-based internal solution was measured at 8.2 mV; however, the data presented were not corrected. All statistics were performed using either the nonparametric tests, Mann–Whitney U test, or Student’s t test except where noted. Average values are reported as means SEM.

**Computational modeling:** A computational model of a VTA dopamine neuron was created in Genesis simulation software (Bower and Beeman, 2007). This model contains a fast sodium current ($Na_f$; Seutin and Engel, 2010; Tucker et al., 2012), a sodium leak current ($Na_L$), a delayed rectifying potassium current (Liu et al., 2012; Khaliq and Bean, unpublished data), an A-type potassium current $I_{A}$, an $I_h$ current (Z. M. Khaliq, unpublished data; Migliore et al., 2008), and the calcium-activated potassium channels, SK and BK (Hirschberg et al., 1998; Maylie et al., 2004; Evans et al., 2013; Jaffe et al. 2011). In addition, the model contains five calcium channels ($T, R, N, L_{1.2},$ and $L_{1.3}$; Tuckwell, 2012; Evans et al., 2013; Table 1). All calcium channels contribute to a calcium pool, modeled with a single time constant of decay (25 ms), which activates the SK and BK channels. The computational model has a spherical soma with two symmetrical primary dendrites which branch into secondary and tertiary dendrites, each dendrite is broken into compartments of 20 µm each for a total of 69 compartments (including soma). All channels are the same density throughout compartments, except the A-type
potassium current, which is limited to the soma and proximal dendrites (50 µm from the soma) to replicate (Gentet and Williams, 2007), and the T-type current, which is located only in the dendrites. The model was tuned to replicate the slow (1–5 Hz) spontaneous firing rate and high input resistance characteristic of dopamine neurons. Voltage-clamp simulations were conducted in a single somatic compartment. The computational model will be made available on ModelDB. Please see Table 1 below for a summary of the conductances used in the model.
Table 1 - Conductance and Permeability Values used in the Dopamine Neuron Model.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Soma</th>
<th>Prox (&lt;51µm)</th>
<th>Dist (&gt;51µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( G_{\text{bar}} , (\text{S/m}^2) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Na} )</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>( \text{Na} , (\text{leak}) )</td>
<td>0.135</td>
<td>0.135</td>
<td>0.135</td>
</tr>
<tr>
<td>( K_{\text{DR}} )</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>( \text{SK} )</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>( \text{BK} )</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>( I_{\text{H}} )</td>
<td>0.5, 1, or 3*</td>
<td>0.5, 1, or 3*</td>
<td>0.5, 1, or 3*</td>
</tr>
<tr>
<td>( K_{\text{A}} )</td>
<td>24.5 to 32*</td>
<td>24.5 to 32*</td>
<td>0</td>
</tr>
<tr>
<td>( P_{\text{bar}} , (\text{cm/s}) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Ca} , \text{-R type} )</td>
<td>0.5e-6</td>
<td>0.5e-6</td>
<td>0.5e-6</td>
</tr>
<tr>
<td>( \text{Ca} , \text{-N type} )</td>
<td>1.2e-6</td>
<td>1.2e-6</td>
<td>1.2e-6</td>
</tr>
<tr>
<td>( \text{Ca} , \text{-L type (1.2)} )</td>
<td>0.1e-6</td>
<td>0.1e-6</td>
<td>0.1e-6</td>
</tr>
<tr>
<td>( \text{Ca} , \text{-L type (1.3)} )</td>
<td>5e-9</td>
<td>5e-9</td>
<td>5e-9</td>
</tr>
<tr>
<td>( \text{Ca} , \text{-T type} )</td>
<td>0</td>
<td>0.1e-6 or 1e-6*</td>
<td>0.1e-6 or 1e-6*</td>
</tr>
</tbody>
</table>

*conductances and permeabilities altered as indicated in Figure 11
2.4 RESULTS

2.4.1 Similar post-burst pauses but larger rebound delays in mesoaccumbal versus nigrostriatal dopaminergic neurons.

Dopaminergic neurons recorded in vivo pause their firing in response to aversive stimuli (Ungless et al., 2004; Matsumoto and Hikosaka, 2009) and reward omission (Schultz et al., 1997; Cohen et al., 2012). Past work analyzing spontaneous burst-pause activity recorded in anesthetized rats reported substantially longer post-burst pauses in mesoaccumbal neurons compared to the nigrostriatal neurons (Clark and Chiodo, 1988). However, whether differences in intrinsic properties can account for the distinctly longer burst pauses reported in mesoaccumbal neurons has yet to be determined. Therefore, we first compared post-burst pauses recorded in midbrain dopamine neurons categorized according to their axonal projections to either nucleus accumbens (mesoaccumbal) or dorsal striatum (nigrostriatal) dopamine subpopulations.

To visually identify dopamine neurons projecting to distinct brain regions, we injected CTB-AF555 into either the nucleus accumbens or dorsal striatum of mice that express GFP driven by the tyrosine hydroxylase promoter (TH-GFP; Matsushita et al., 2002; Fig. 1). Injections into the nucleus accumbens resulted in a majority of labeled neurons located in the ventral tegmental area (Fig. 1A–C). While most dual labeled neurons were present in VTA, many could be found at the border of VTA and SNc with occasional neurons found in the SNc. Similarly, we found that CTB injections into the dorsal striatum resulted in dual labeled neurons in the SNc with a number of neurons found at the border of SNc and VTA (Fig. 1D–F). Because the borders of these two nuclei (SNc and VTA) are not well defined and contain mixtures of mesoaccumbal and nigrostriatal neurons, retrograde labeling of dopamine neuron subpopulations was necessary for clear identification of cell types. Therefore, whole-cell recordings were made only from neurons co-labeled with GFP and CTB-AF555, indicating projection-specific dopamine neuron subpopulations.
To test post-burst pauses, we interrupted spontaneous firing with 1 s depolarizing current pulses covering a range of amplitudes to evoke high-frequency firing, and then measured the subsequent post-burst pause (Fig. 2C, D). We found that the rates of spontaneous firing in mesoaccumbal and nigrostriatal neurons were comparable at 2.89 ± 0.14 Hz (n = 89) versus 2.43 ± 0.14 Hz (n = 72; Mann–Whitney test, p = 0.03) but statistically different.

Comparing burst pauses at maximal firing rates, we found that mesoaccumbal dopamine neurons exhibited substantially higher maximal firing rates at 20.36 ± 1.54 Hz, resulting in longer post-burst pauses of 1.92 ± 0.18 s (n= 28) relative to nigrostriatal neurons, which fired maximally at 13.18 ± 0.8 Hz, resulting in an average post-burst pause of 1.3 ± 0.09 s (n = 36). However, when comparing pauses following bursting at similar rates, we found that the duration of post-burst pauses was similar between mesoaccumbal and nigrostriatal neurons (Fig. 2D). In contrast to previous work in vivo (Clark and Chiodo, 1988), we found that the intrinsically generated post-burst pauses do not differ between mesoaccumbal and nigrostriatal neurons. A separate variety of pauses occurs as a result of stimuli that directly inhibit firing, such as those originating from activation of inhibitory GABA$_A$ mediated inputs (Tepper and Lee, 2007), or from activation of hyperpolarizing modulatory receptors such as GABA$_B$ receptors (Edwards et al., 2017) or dopamine D2 autoreceptors (Gantz et al., 2013). To gain insight into the intrinsic cell properties that contribute to pauses resulting from hyperpolarizing inhibition, we next examined rebound properties of dopamine neurons. We interrupted spontaneous firing with a range of hyperpolarizing current injections and measured the latency to the first spike as the cell resumed firing, hereafter called the “rebound delay” (Fig. 2E, F, I). We then plotted the rebound delay against the cell’s voltage measured at the point that the current injection was released, referred to as the “baseline voltage” (Fig. 2F, I). There was substantial variability within dopamine neuron subpopulations, with rebound delays ranging from 128.8 ms to 1.3 s in mesoaccumbal neurons and 99 to 882.9 ms in nigrostriatal neurons (measured from a baseline of 65 mV to 75 mV; Fig. 2I, J). Interestingly, however, the average duration of rebound delays
were twice as long in mesoaccumbal neurons as compared to nigrostriatal neurons (maximal rebound delays at -75 mV, mesoaccumbal, $650.8 \pm 100.83 \text{ ms, } n = 24$; nigrostriatal, $327.8 \pm 44.6 \text{ Hz, } n = 31$; Mann–Whitney test, $p = 0.0038$; Fig. 2F). As a complementary measurement of the delay, we measured the depolarizing slope of the membrane potential during the rebound delay, called the “rebound slope.” We found that a plot of the rebound slope against the rebound delay was fit well by an exponential function in both the mesoaccumbal and nigrostriatal subpopulations (Fig. 2H, J). In particular, we found that steeper rebound slopes and, by consequence, faster rebound delays were more likely present in the nigrostriatal dopamine neurons. Together, our results show that mesoaccumbal and nigrostriatal neurons exhibit similar spontaneous activity and post-burst pauses, but display divergent responses to hyperpolarization as shown in the differences in their rebound properties.

2.4.2 Development of rebound delays evoked by a range of brief duration hyperpolarizations.

We next quantified the dependence of the rebound delay on the duration of the hyperpolarizing current for injections ranging from 1 to 500 ms. An example recording from a mesoaccumbal neuron is provided in Figure 3A. We found that brief hyperpolarization durations of 1, 5, or 10 ms had little effect on the timing of subsequent spikes. However, longer hyperpolarizations of 25 ms and above resulted in substantial increases in rebound delays, with the maximal rebound delay of 711.9 ms occurring in response to a 100 ms hyperpolarization. Longer hyperpolarizations (100 ms) produced no further enhancement of the delay. Similarly, in the example, nigrostriatal neuron shown in Figure 3B, a hyperpolarization of 100 ms resulted in a maximal delay of only 266.2 ms. We observed the same trend across a population of cells (Fig. 3C, D). Mesoaccumbal neurons displayed average maximal rebound delays of $1207 \pm 186 \text{ ms (n = 16)}$ with 14 of 16 cells achieving maximal delays following hyperpolarizing pulses of 100 ms. In nigrostriatal neurons, maximal delays were achieved with following hyperpolarizing pulses of 50 ms in 2 neurons, 100 ms in 4 neurons, and 500 ms in 1 neuron. Among these
neurons, the duration of the maximal rebound delay was an average of 578.12 ± 93.65 ms (n = 7; Mann–Whitney test, p 0.047). Therefore, although mesoaccumbal and nigrostriatal neurons differ in the duration of their maximal delays, these results demonstrate that an initial hyperpolarization of 100 ms in duration is sufficient to reach maximal rebound delay in both cell types.

2.4.3 Effect of subthreshold inhibitory current pulses on spike timing during pacemaking

Long-duration, severe hyperpolarizations are typically used to evoke rebound delays. Although convenient experimentally, these hyperpolarizations may recover channels that would have been otherwise inactivated and may exaggerate the contribution of these conductances to excitability within the physiological voltage range. Therefore, we focused on firing responses to short hyperpolarizations (5, 25, and 100 ms) designed to mimic activity from a single input or short summating bursts of hyperpolarizing inhibitory inputs. To test this, we injected hyperpolarizing current injections of increasing amplitudes on a background of spontaneous firing activity. Figure 4A provides the layout of the experiment wherein increasing amplitudes of current injections that lasted 5, 25, and 100 ms were injected, interleaved by spontaneous firing activity in both mesoaccumbal and nigrostriatal dopamine neurons. Figure 4, B and C, provides typical responses to 100 ms current injections in mesoaccumbal and nigrostriatal neurons. A current injection that hyperpolarized neurons to near −55 mV resulted in a 294.8 ms delay in the mesoaccumbal neuron, but only slightly shorter delay of 285.2 ms in the nigrostriatal neuron. Further hyperpolarization to near −65 mV, however, resulted in a delay of 522.6 ms in mesoaccumbal neurons, but a somewhat shorter delay of 420.2 ms in the nigrostriatal neuron.

We next quantified the effect of brief current injections on spike timing. To do this, we normalized the duration of the interspike interval affected by the current injection (ISI$_0$) to the preceding interspike interval during spontaneous firing (ISI$_1$), calculated as the ISI$_1$/ISI$_0$ ratio (Fig. 4B,C). To robustly compare effects across neurons and reduce variability in the timing of
the onset of the current injections, we analyzed injections that occurred only within the middle
50% of the interspike interval (ISI). In our example cells, hyperpolarizing to a minimum voltage
of −65 mV for 100 ms in the mesoaccumbal neuron resulted in an ISI$_1$/ISI$_0$ ratio of 2.10, an
increase of more than double the duration of the control interspike interval. In the nigrostriatal
neuron, however, we observed a ISI$_1$/ISI$_0$ ratio of only 1.5, a 50% increase in the interspike
interval.

In a population of cells, we plotted the ISI$_1$/ISI$_0$ ratio against the minimum voltage
reached following the hyperpolarizing current injection. We reasoned that plotting the minimum
voltage should control for potential differences in input resistance between cell types. Following
100 ms current injections that hyperpolarized cells to −55 mV, we found an ISI$_1$/ISI$_0$ ratio of 1.71
± 0.08, a 70.8% increase in the interspike interval of mesoaccumbal neurons ($n = 16$),
compared to an ISI$_1$/ISI$_0$ ratio of 1.44 ± 0.09, an increase of the 44% in nigrostriatal neurons ($n =
8$; $p = 0.048$; Fig. 4F). With further membrane hyperpolarization to −65 mV, well within
physiological voltage range in dopamine neurons, the difference between ISI$_1$/ISI$_0$ ratios
measured in mesoaccumbal and nigrostriatal neurons became more prominent. The
ISI$_1$/ISI$_0$ ratio following hyperpolarization to −65 mV in mesoaccumbal neurons was 2.36 ± 0.1,
an increase in duration of 136%, and in nigrostriatal neurons it was 166.2 ± 0.04, an increase in
duration of 66.2% ($n = 8$; $p = 3.2e-7$). Probing the response to 5 and 25 ms hyperpolarizations,
however, we found no difference in the ISI$_1$/ISI$_0$ ratio between subpopulations at any voltage
measured (Fig. 4D,E). Collectively, these data demonstrate that mesoaccumbal and
nigrostriatal neurons differ substantially in their sensitivity to both long (1 s) and short (100 ms)
hyperpolarizing inputs, even for hyperpolarizations that occur near physiological voltages that
are reached during natural pacemaker firing.

To rule out the possibility that these subpopulation specific responses may be attributed
to differences in passive properties, we calculated the resistance in each neuron that resulted
from the current amplitude injected in both mesoaccumbal and nigrostriatal dopamine neurons
Current injections that occurred only during the middle 50% of the interspike interval were used to calculate the resistance. To compare input resistance between mesoaccumbal and nigrostriatal cells, we averaged calculated resistance values over a range of current steps for which the resistance values reached steady state (Fig. 4G–I, gray bars; 5 ms steps, 200–500 pA; 25 and 100 ms steps, 40–120 pA). Comparing the averaged resistance calculated at steady state for each neuron, we found that there was no significant difference between mesoaccumbal and nigrostriatal neurons in the resistance values for steps of 5 ms ($p = 0.49$), 25 ms ($p = 0.70$), and 100 ms ($p = 0.50$). This confirms that the differences in spike-to-spike latency that we observed between mesoaccumbal and nigrostriatal neurons are not due to differences in passive membrane properties.

### 2.4.4 Hyperpolarization-evoked spiking delays are lengthened by a subclass of subthreshold potassium current ($I_A$).

Previous studies have shown that A-type potassium currents comprise the dominant subthreshold outward current in dopamine neurons, constraining the rate of spontaneous firing in VTA (Koyama and Appel, 2006; Khaliq and Bean, 2008) and SNc dopamine neurons (Liss et al., 2001). In addition, A-type potassium currents are known to contribute to the duration of the rebound delay in SNc cells (Amendola et al., 2012). We next tested the relationship between the interspike voltage trajectory and rebound delay within cells in mesoaccumbal and nigrostriatal subpopulations (Fig. 5A,B). Plotting the interspike voltage slope against the rebound delay, we found that the interspike slope shows a moderate to strong negative correlation with the duration of the rebound delay in both the mesoaccumbal neurons ($Pr = -0.64, n = 20, p = 0.0024$) and nigrostriatal neurons ($Pr = -0.62, n = 28, p$-value $= 0.00043$) (Fig. 5C,D). This demonstrates that despite the large cell-to-cell variability that exists within the data sets from each subpopulation, a clear relationship exists between the duration of the rebound delay and the voltage trajectory of the interspike spike interval in spontaneously firing dopamine neurons.
We next tested the contribution of A-type potassium currents to the interspike voltage trajectory of mesoaccumbal and nigrostriatal neurons using the specific A-type potassium channel blocker, AmmTX3 (Vacher et al., 2002). Bath application of 0.5 – 1μM AmmTX3 resulted in a speeding of rate of spontaneous firing by 54% (control, 3.0 ± 0.2 Hz; AmmTX3, 4.7 ± 0.3, n = 10) in mesoaccumbal neurons, and by 76% (control, 2.8 ± 0.4 Hz; AmmTX3, 4.9 ± 0.5, n = 5) in nigrostriatal neurons. In addition, we compared the voltage trajectory of the interspike intervals. On average, we found a significant increase in the slopes of the interspike interval in response to AmmTX3 in mesoaccumbal (control, 38.7 ± 4.0 mV/s; AmmTX3, 65.3 ± 8.7 mV/s; n = 10) and nigrostriatal neurons (control, 39.0 ± 7.0 mV/s; AmmTX3, 98.4 ± 10.4 mV/s; n = 5; p = 0.0015; Fig. 5E,F). Supporting previous findings (Khaliq and Bean, 2008), therefore, our results are consistent with the idea that A-type potassium currents control pacemaking by dynamically controlling the rate of spontaneous depolarization during the interspike interval.

We next tested the contribution of A-type potassium currents to rebound delays in the mesoaccumbal and nigrostriatal dopamine neuron subpopulations (Fig. 5G, H). We found that bath-applied AmmTX3 dramatically reduced the duration of rebound delays to only a fraction of the control duration in both mesoaccumbal (percentage control in AmmTX3, 79%; n = 9; p = 0.001; Fig. 5I) and in nigrostriatal neurons (percentage control, 83%; n = 6; p = 0.01; Fig. 5J). Altogether, these data provide a clear demonstration that the variability in rebound delays that we observe across mesoaccumbal and nigrostriatal dopamine neuron subpopulations correlates strongly with subthreshold voltage trajectory, suggesting that \( I_A \) is the common link between rebound delay and interspike voltage trajectory.

### 2.4.5 GABA-evoked pauses are prolonged by recruitment of A-type potassium currents.

Our results so far strongly suggest that A-type potassium currents prolong the duration of spiking delays following somatic current injections but do not directly address their role in
synaptically evoked inhibitory pauses. Therefore, we tested the effect of AmmTX3 on GABAergic pauses evoked by 50 Hz stimulation of inhibitory inputs for 300 ms. To isolate GABAergic inputs, we included in the bath solution 50 μM D-AP5 and 20 μM CNQX to block NMDA and AMPA receptors along with the nonspecific mGluR antagonist 1 μM LY341495 and 1 μM sulpiride to block dopamine (D₂) autoreceptors. Following a 10 min baseline period, we found that bath application of AmmTX3 resulted in a 56% reduction of the averaged pause, from 1783 ± 44 ms in control to 789 ± 16 ms in AmmTX3 (n = 6; Fig. 6A,B). To verify that the stimulation-evoked pause was synaptic in nature and not due to direct stimulation, we blocked GABAₐ and GABAₜ receptors using 100 μm picrotoxin and 1 mm CGP55845. This manipulation completely abolished the pause, as shown in Figure 5A (blue trace).

In an analogous set of experiments, we evoked pauses in dopamine neurons by shining blue light to uncage RuBi-GABA (40 μm) applied to the circulating bath solutions. Similarly, we found that GABA uncaging-evoked pauses were significantly shortened by 42% in the presence of AmmTX3, from 1995 ± 120 ms in control to 1164 ± 83 ms in AmmTX3 (n = 6; Fig. 6C,D). These uncaging experiments support our above findings examining synaptically generated pauses and rule out the possibility that the effect of AmmTX3 could be presynaptic. Therefore, these results demonstrate that A-type potassium currents are recruited during active inhibitory neurotransmission and likely prolong the duration of GABAergic pauses.

2.4.6 Comparison of inactivation kinetics, voltage dependence, and recovery of A-type potassium currents in mesoaccumbal and nigrostriatal neurons.

To better understand the ionic basis of the differences observed in the rebound delay between mesoaccumbal and nigrostriatal subpopulations, we performed voltage-clamp experiments to test the biophysical properties of the A-type potassium currents. To isolate A-type potassium currents, we recorded in a cocktail of blockers that included 1 μM TTX, 30 μM nifedipine, and 50 mM TEA-Cl to block voltage-gated sodium currents, low-threshold L-type Ca2+ currents, and high-threshold potassium currents.
We first measured the kinetics and voltage dependence of inactivation of isolated A-type potassium currents. Currents were evoked by steps from −90 to −40 mV. A-type potassium currents in both mesoaccumbal and nigrostriatal neuron subpopulations were by far the largest amplitude currents that were present in the dopamine neurons (Liss et al., 2001; Khaliq and Bean, 2008; Amendola et al., 2012; Figs. 7A,B). The amplitude of $I_A$ measured from steps to −40 mV did not differ significantly between subpopulations (mesoaccumbal, 1.65 ± 0.32 nA, $n = 22$; nigrostriatal, 1.3 ± 0.15 nA, $n = 18$; Mann–Whitney test, $p = 0.26$). However, we observed a striking difference in the inactivation kinetics of the A-type currents between mesoaccumbal and nigrostriatal subpopulations (Fig. 7C,D). Specifically, A-type currents recorded in nigrostriatal neurons decayed with relatively fast time constants ranging from 13.5 ms to 68.9 ms, and exhibited an average decay time constant of 36.2 ± 3.3 ms ($n = 18$; Fig. 7D). By contrast, A-type currents measured in mesoaccumbal neurons displayed a much wider range of inactivation time constants from 14.5 to 224.3 ms, with 11 of 22 cells exhibiting atypically slow inactivation kinetics with decay time constants longer than 100 ms. On average, A-type currents in mesoaccumbal neurons had substantially slower decay time constants of 98.7 ± 10.8 ms ($n = 22$; Fig. 7D; mesoaccumbal vs nigrostriatal, $p = 3.4e^{-05}$, Mann–Whitney test). As a point of comparison, A-type currents measured in outside-out patches from CA1 pyramidal cells decay much faster, with an average time constant of 26 ms (Kim et al., 2008). Therefore, we found that inactivation of A-type potassium currents in dopamine neurons occurs over a broad range of inactivation rates, but is unusually slow in mesoaccumbal neurons.

In the same set of cells, we compared the voltage dependence of inactivation. We determined the voltage dependence by plotting the peak current measured at −40 mV against the prepulse voltage and fit the data to the Boltzmann equation to obtain voltages of half-inactivation. Interestingly, we found that the voltage of half-inactivation of A-currents occurred at slightly more hyperpolarized potentials in mesoaccumbal neurons relative to nigrostriatal neurons. On average, half-inactivation voltage was −66.6 ± 1.4 mV (slope, 5.2 ± 0.4; $n = 18$) in
mesoaccumbal neurons but $-62.7 \pm 0.7$ mV (slope, $4.7 \pm 0.16$; $n = 22$) in nigrostriatal neurons (mesoaccumbal vs nigrostriatal, $p = 0.02$, Mann–Whitney U; Fig. 7E).

It is possible that the range of kinetics observed in our recordings may result partly from inadequate space clamp. However, two pieces of evidence argue against this scenario. First, outside-out patch recordings from the dendrites and soma of SNc dopamine neurons indicate that the highest of density of $I_A$ channels is located in the soma (Gentet and Williams, 2007). Second, dopamine neurons exhibit extraordinarily high input resistances of 0.3–1 GΩ for principal neurons and their dendrites display little branching, both of which result in strongly isopotential soma and proximal dendrites (Hausser et al., 1995; Khaliq and Bean, 2010; Hage and Khaliq, 2015).

The roughly exponential time course of the development of the rebound delay shown in Figure 2 hints strongly at a process that depends upon recovery from channel inactivation. Therefore, we compared recovery from inactivation of A-type currents recorded from mesoaccumbal and nigrostriatal dopamine neurons (Fig. 8). To test recovery from inactivation, we applied a 250 ms conditioning step to $-40$ mV to allow for nearly complete inactivation of $I_A$ that was then followed by a variable period of recovery at $-70$ mV (Fig. 8A). The extent of recovery was then assayed using a test pulse to $-40$ mV. Plotting the peak of the test pulse versus the recovery time period, we found that these values fit well to a single exponential indicating that recovery occurs mainly from only a single inactivated state (Fig. 8B). In a population of cells (Fig. 8C), the time constant of recovery recorded in mesoaccumbal neurons ranged widely from 24.26 to 133.33 ms, with an average of $64.51 \pm 12.84$ ms ($n = 8$). In nigrostriatal neurons, there was a trend toward a slightly quicker recovery from inactivation which ranged from 32.1 to 46.28 ms, with an average of $36.92 \pm 1.86$ ms ($n = 7$). These values for recovery from inactivation in both cell types match approximately the values for development of the rebound delay shown in Figure 2, further suggesting that A-type currents contribute strongly to rebound delays. Therefore, we found that mesoaccumbal and nigrostriatal dopamine
neurons, exhibited pronounced differences in the kinetics and voltage dependence, but not amplitudes, of their A-type potassium currents.

2.4.7 Comparison of $I_H$ and sag potentials in mesoaccumbal and nigrostriatal neurons

Because hyperpolarization-gated cation currents ($I_H$) are known to reduce the duration of the rebound delay in midbrain dopamine neurons (Neuhoff et al., 2002; Amendola et al., 2012), we next asked whether differential expression of $I_H$ contributes to the differences in rebound delays observed between mesoaccumbal and nigrostriatal dopamine neurons. We directly recorded $I_H$ in voltage clamp with 1 s voltage steps from $-40$ mV to a range of hyperpolarized voltages between $-80$ and $-120$ mV (Fig. 9A, B). On average, the amplitude of $I_H$ was significantly larger in the nigrostriatal compared to mesoaccumbal neurons (amplitude at $-120$ mV, mesoaccumbal, $-257.9 \pm 56.3$ pA, $n = 12$; nigrostriatal, $-577.3 \pm 50.7$, $n = 9$; $p = 0.0033$; Fig. 9C). Quantifying the kinetics of $I_H$, however, we found that $I_H$ activation is slow. The activation time constant measured from steps to $-100$ mV was $\sim 1$ s, and we observed no significant differences in the average activation time constant for $I_H$ in mesoaccumbal and nigrostriatal dopamine neurons at any of the voltages that were tested (Fig. 9D).

Dopamine neurons respond to hyperpolarizing current injection with a characteristic depolarizing voltage sag due to activation of $I_H$ (Fig. 9E, F). We next compared sag potentials in dopamine neuron subpopulations. To do this, we evoked voltage sags by injecting hyperpolarizing current and then quantified sag potentials by taking the voltage difference between the trough voltage and the steady baseline voltage at the end of the current step. Consistent with our voltage-clamp results described above, we observed that voltage sags were approximately twice as large in nigrostriatal neurons as measured in mesoaccumbal neurons (sag potential at baseline of $\sim-86$ mV, mesoaccumbal, $14.1 \pm 2.05$ mV, $n = 28$; nigrostriatal, $29.3 \pm 3.04$ mV, $n = 36$; $p = 0.00052$; Fig. 9G). Together, our results demonstrate the presence
2.4.8 Correlating A-type potassium current kinetics and I_H amplitude to the
duration of rebound delay in mesoaccumbal and nigrostriatal dopamine neurons.

Because mesoaccumbal and nigrostriatal dopamine subpopulations differ in the kinetics
of A-type potassium currents and in the amplitude of I_H, we next asked how closely the
differences in conductances correlate with the observed neuronal responses to
hyperpolarization (Fig. 10A, B). First, plotting the rebound slope and against the inactivation
kinetics of the A-type potassium currents (Fig. 10C), we found a strong negative correlation
between rebound slope and the time constant of A-type current inactivation (Pr = −0.69, n =
29, p = 3.56e-0.5). We next compared values for rebound slope and the amplitude of the
I_H current and found that these values were positively correlated with a Pr value of 0.59 (p =
0.0016; Fig. 10D). We also found a correlation between the amplitude of A-type current (at −40
mV) and the amplitude of I_H (at −120 mV; Pr = 0.5, n = 26; p = 0.009; Fig. 10E). By contrast, we
found little to no correlation between the rebound slope and absolute amplitude of the A-type
potassium current (Pr = −0.13; Fig. 10F). Therefore, our present data in the identified
mesoaccumbal and nigrostriatal subpopulations shows that the kinetics of I_A and the amplitude
of I_H correlates well with the duration of rebound delays in dopamine neurons.

2.4.9 Testing the relative contribution of I_A kinetics and I_H amplitude to rebound
delay in a computational dopamine neuron model

To develop a better understanding of how differences in I_A kinetics and I_H amplitude
contribute to the rebound delay, we constructed generalized single and multi-compartmental
computational models of a midbrain dopamine neuron containing ionic conductances that
approximated those recorded in our cells. The model enabled us to selectively alter only the
time constant of I_A inactivation while preserving the peak current amplitude, a manipulation that
is not experimentally possible, and to generate a within-cell prediction of how this manipulation
would influence rebound delays and GABAergic pauses. Figure 11A shows traces of simulated A-type currents with inactivation decay time constants ranging from 25–200 ms in a single compartmental model of a dopamine neuron soma. We incorporated these conductances separately into the multi-compartmental model and ran simulations of the rebound delay or inhibitory pauses as shown in the traces provided in Figure 11B–D.

Our first simulations tested the effect of slowing $I_A$ inactivation in a mesoaccumbal-like model cell with only a small amplitude $I_H$ (Fig. 11B,C). Consistent with the correlations from our experimental results, simulations of the mesoaccumbal-like model predicted that slowing the time constant of inactivation, while maintaining the peak current amplitude at a fixed value, is sufficient to lengthen the pause in firing following hyperpolarization. In particular, an inactivation time constant of 25 ms resulted in a rebound delay of 179.13 ms, while a decay time constant of 200 ms resulted in a delay of 1.038 s, about six times longer. Similarly, we tested the direct effects of changing inactivation kinetics of $I_A$ on the duration of pauses that follow GABAergic stimulation (Fig. 11D). We simulated a 50 Hz GABAergic stimulation (300 ms) onto a background of spontaneous firing in the model cell with inactivation decay values ranging from 25 to 200 ms. We found that for an $I_A$ decay time constant of 25 ms, the spike-to-spike delay was 604.98 ms, compared to 1.35 s for an $I_A$ time constant of 200 ms (Fig. 11D). As such, our model shows that the decay time constant of $I_A$ is a direct contributor to pauses that result from synaptic inhibition.

However, it is possible that a model cell with a relatively large $I_H$ amplitude (e.g., a nigrostriatal-like model) may be less affected by changes in the $I_A$ inactivation time constant. Interestingly, simulations of a nigrostriatal-like model neuron, which had a substantially larger $I_H$ (two or six times larger amplitude, as indicated by the prominent sag shown in Fig. 11E) were qualitatively similar to the model with a small $I_H$ (1X). We found that increasing the density of $I_H$ in the model cell speeded the rebound delay in a mesoaccumbal-like model neuron with 200 ms $I_A$ inactivation time constant (Fig. 11E). Rebound delays for model cells with $I_H$ densities of 1,
2, and 6X were 1038.14, 964.97, and 799.66 ms. Therefore, increasing the density of $I_H$ by six times resulted in a reduction in the slope of the rebound delay versus inactivation time constant relationship (Fig. 11F). However, increasing the amplitude of $I_H$ did not abolish the effect of slowing inactivation on the rebound delay. In combination with the relative amplitude of $I_A$ and $I_H$, these simulations from our computational model support correlations from our experimental data that the kinetics of $I_A$ strongly regulate the timing of spikes following hyperpolarizing inhibition.

Midbrain dopamine neurons have been shown to express T-type calcium currents (Kang and Kitai, 1993; Wolfart and Roeper, 2002; Philippart et al., 2016), which are also activated in approximately the same voltage range as A-type potassium currents (Anderson et al., 2010). Therefore, we assessed the effect of T-type calcium currents on the rebound delay in our model neuron. Increasing the T-type calcium conductance from 1X to 10X greater resulted in a decrease in the rebound delay for all $I_A$ inactivation time constants examined (Fig. 11G,H). However, we found that increasing T-type currents did not significantly alter the overall trend in our data. Therefore, our findings are consistent with the idea that T-type calcium currents contribute to shorter rebound delays, but are less effective in the presence of slowly inactivating $I_A$. Together, computational modeling and experimental results clearly demonstrate the role of slow inactivation kinetics of $I_A$ in shaping the rebound pause.

2.5 DISCUSSION

Here, we show that mesoaccumbal and nigrostriatal dopamine neurons are distinguished by their sensitivity to inhibitory events (‘rebound properties’) resulting from differences in the intrinsic conductances that shape pauses. In particular, we found that mesoaccumbal neurons respond to hyperpolarizing current pulses with significantly longer delays in spiking relative to nigrostriatal neurons. The differences in the rebound delays were dependent upon the strength and duration of inhibitory pulses, with hyperpolarizations of 100 ms or longer evoking near maximal delays in both neuronal subpopulations. The ionic...
The mechanism underlying longer delays in mesoaccumbal neurons is an inactivating A-type potassium current that decays slowly, along with weak expression of H-currents. Computational modeling supports the idea that A-type potassium currents are recruited during rebound delays and synaptically-evoked pauses to prolong pauses in a manner that strongly depends on the inactivation time constant of $I_A$. Altogether, our results demonstrate that $I_A$ shapes responses of dopamine neurons to GABAergic inhibitory inputs and suggest that the slow kinetics of $I_A$ result in heightened sensitivity of mesoaccumbal neurons to hyperpolarizing inhibition. We propose that these results may explain, in part, the observation that many dopamine neurons of the VTA and ventromedial SNc recorded in vivo exhibit robust pause responses to aversive stimuli while dorsolateral SNc neurons exhibit much weaker responses (McHaffie et al., 2006; Brown et al., 2009; Matsumoto and Hikosaka, 2009; Lerner et al., 2015).

### 2.5.1 Heterogeneity in $I_A$ inactivation kinetics - molecular mechanism.

Heterogeneity in $I_A$ inactivation kinetics has been observed in several neuronal types. Specifically, decay time-constants ranging from 20 ms to 100 ms, and even up to 300 ms, have been observed in neurons of the nucleus of the tractus solitarii (Strube et al., 2015), tuberomammillary neurons (Jackson and Bean, 2007), globus pallidus and basal forebrain neurons (Baranauskas, 2004). Likewise, our data show a broad range of inactivation kinetics in mesoaccumbal neurons with many cells exhibiting atypically slow inactivating A-type potassium currents (average tau inactivation of mesoaccumbal neurons, ~98 ms) while mesostriatal neurons display a much narrower range of inactivating currents, consistent with data from unlabeled SNc dopamine neurons (Liss et al., 2001; Amendola et al., 2012). By contrast, A-type potassium currents found in the hippocampal CA1 pyramidal and most cerebellar granule neurons inactivate more quickly with decay time constants between 10 ms and 45 ms (Jerng et al., 2004b; Kim et al., 2008).

The molecular mechanism of heterogeneity among A-type potassium currents has been
examined both in heterologous expression systems and in a variety of neuronal cell types (Carrasquillo et al., 2012; Jerng and Pfaffinger, 2014). From these studies, several accessory subunits have been identified and shown both to influence channel density and to modulate the properties of A-type currents, including dipeptidyl peptidase (DPP) subunits (Nadal et al., 2003) as well as the Kv channel-interacting proteins (KChIP) (An et al., 2000; Ohya et al., 2001). A rigorous study of A-type currents in pyramidal neurons of the visual cortex employing knockout models and RNAi knockdown techniques found that KChIP2, KChIP3, and KChIP4 are necessary for the formation and expression of functional Kv4.2 channels (Norris et al., 2010). A separate study of globus pallidus and basal forebrain neurons found a correlation between the presence of KChIP4 subunit A mRNA and slowly inactivating A-type currents (up to 300 ms inactivation time-constant) (Baranauskas, 2004). These studies are consistent with results from expression systems showing that co-expression of KChIP1-4 with Kv4 subunits slows inactivation and speeds the recovery from inactivation (Patel et al., 2002; Gebauer et al., 2004; Jerng et al., 2005; Kitazawa et al., 2014).

In dopamine neurons, the molecular correlate of I_A remains an important and open question. Past work in substantia nigra neurons has shown that the pore forming subunit, Kv4.3, exists within a complex with the long isoform of KChIP3 (Liss et al., 2001). Interestingly, analysis of a recently published dataset profiling the total cellular RNA of dopaminergic neurons using translating ribosome affinity purification techniques (TRAPseq) in transgenic mice shows results that are consistent with this observation (Brichta et al., 2015) and in addition, reveals high levels of mRNA fragments from DPP6 and DPP10, KChIP1, KChIP3, and KChIP4 in samples from both VTA and SNc dopaminergic neurons. These data align in part with reports that I_A inactivation is speeded in VTA dopamine neurons recorded in KChIP4 knockout mice (Kashiotis, 2011, SfN abstract), which also exhibit fewer and shorter duration pauses in behavioral experiments (Costa, 2014; Costa, 2016). Future work should address the
role KChIPs and other $K_v$ 4 accessory subunits in modulating $I_A$ and further examine the effects of this modulation on the function of distinct dopamine neuron subpopulations.

2.5.2 Diversity of pauses and underlying mechanisms in midbrain dopamine neurons

The two main types of pauses that have been observed in dopamine neurons recorded from *in vivo* preparations are inhibitory pauses and post-burst pauses. Unlike the findings from *in vivo* experiments (Clark and Chiodo, 1988), however, our data show that mesoaccumbal and nigrostriatal dopamine neurons exhibit almost identical post-burst pauses. This observation suggests that the mechanisms that underlie post-burst pauses may be distinct from those shaping inhibitory pauses. Post-burst pauses may involve small-conductance, calcium-activated (SK) currents activated by increases in intracellular $Ca^{2+}$ which result from high-frequency spiking or activation of metabotropic glutamate receptors (Fiorillo and Williams, 1998; Morikawa et al., 2003). In addition, it is likely that bursting neurons can experience pauses caused in part by $Na^+$ channel inactivation during the refractory period (Tucker et al., 2012). Inhibitory pauses are also initiated by synaptic or modulatory inputs (Fiorillo and Williams, 1998; Gantz et al., 2013). However unlike post-burst pauses, our data show that pauses evoked by synaptic inhibition involves far fewer intrinsic conductances and are dominated by $I_A$ and $I_{H}$, but also may involve T-type Ca2+ channels in a subset of SNc neurons (Evans, 2015). Therefore, the intrinsic conductances that differentiate pauses in mesoaccumbal and nigrostriatal neurons are valid for inhibitory pauses. The extent to which these conductances contribute to pauses evoked by excitation remains undetermined.

2.5.3 Functional Significance

A number of recent studies *in vivo* have demonstrated physiological heterogeneity in responses to aversive stimuli that correlates with the anatomical position of cells within the midbrain. For example, a study of dopamine neurons in monkeys reported that dorsolateral tier SNc neurons (dorsal striatum projecting) increase their firing in response to aversion, while
ventromedial SNc and VTA neurons (ventral striatum projecting) pause their activity (Matsumoto and Hikosaka, 2009). In a separate study in mice, intracellular Ca2+ signals recorded in response to aversive stimuli were enhanced in dorsolateral striatum projecting dopamine neurons but inhibited in ventromedial striatum projecting neurons (Lerner et al., 2015). Similarly, other studies have shown that aversive stimuli result in either short-latency pauses or only weakly reduce firing in SNc neurons (Brown et al., 2009), but result in long-latency aversive pauses in VTA dopamine neurons (Mileykovskiy and Morales, 2011). While these studies establish that diversity exists in aversive responses among dopamine neurons, our results provide insight into the underlying cellular mechanisms. To build upon our findings of diversity in integration of inhibitory inputs, it will be important for future experiments performed in vitro and in vivo to examine features of synaptic inputs, including determining activity patterns of presynaptic neurons and synaptic weights that contribute to overall functional heterogeneity among dopamine neurons.
FIGURES

Nucleus Accumbens → Mesoaccumbal (MA)

A

C

Dorsal Striatum → Nigrostriatal (NS)

D

E

F

TH-GFP
CTB-AF555

TH-GFP
CTB-AF555

TH-GFP
CTB-AF555
Figure 2-1 Retrograde labelling of mesoaccumbal and nigrostriatal dopamine neurons.

**A**, Schematic of a sagittal section of a mouse brain along with mesoaccumbal projection. CTB conjugated to Alexa555 was injected into nucleus accumbens to retrogradely label dopamine neurons in the VTA. **B**, Schematic (left) and 10× tiled image of a coronal section of midbrain slice (right) in TH-GFP mouse depicting mesoaccumbal subpopulations. GFP-positive TH cells are show in green, and CTB-positive NAc-projecting neurons are shown in red. **C**, Image of VTA and SNc demonstrating the mesoaccumbal subpopulation. **D**, Schematic of injection site of CTB in the dorsal striatum. **E**, Schematic (left) and 10× tiled image (right) of a coronal section of midbrain slice in same TH-GFP mouse depicting nigrostriatal subpopulation. **F**, Image of the VTA (medial) and SNc (lateral). DS, Dorsal striatum; VS, ventral striatum.
Figure 2-2 Comparison of post-burst pauses and rebound delays in mesoaccumbal and nigrostriatal dopamine subpopulations.

A, Injection site of CTB in the nucleus accumbens (left) and dorsal striatum (right) of a TH-GFP mouse. B, Example of TH-GFP+ dopamine neuron retrogradely labelled with CTB-AF555 injected into nucleus accumbens. C, Example traces in which spontaneous firing recorded in mesoaccumbal (blue) and nigrostriatal (black) neurons has been interrupted with a depolarizing current injection. Arrows indicate post-burst latency. D, Plot of averaged binned post-burst latency versus burst frequency in mesoaccumbal and nigrostriatal dopamine neurons. Closed symbols represent the mean ± SEM. Lines represent data from individual neurons (blue, mesoaccumbal; grey, nigrostriatal). E, Example traces of rebound delays from mesoaccumbal (blue) and nigrostriatal (black) in response to a 1 s hyperpolarizing current injection. Baseline voltage values are indicated. F, Plot of averaged binned maximal rebound delays versus baseline voltage. G, Examples of rebound delays shown on an expanded scale for clarity showing linear fit of the rebound delay (red lines) as a measure of the rebound slope. H, Plot of rebound slope versus rebound delay in mesoaccumbal dopamine neurons (triangles) measured from a baseline voltage of ∼−75 mV. The red line indicates exponential fit. I, Summary of rebound pause duration in mesoaccumbal neurons (left). Dark blue lines show individual neurons (N = 24), and light blue circles show 5 mV binned averages ± SEM. A summary of nigrostriatal neurons is shown on the right. Grey lines show individual neurons (N = 31), and black circles and lines show 5 mV binned averages ± SEM. J, Same plot as in H but in nigrostriatal dopamine neurons (circles). The red line indicated exponential fit. Asterisks indicate a P value <0.05.
**Figure 2-3 Time-dependent development of the rebound delay.**

**A**, Example of rebound pauses in a mesoaccumbal neuron in response to 120 pA hyperpolarizing current injection delivered for different durations. **B**, Same as in A for an example nigrostriatal neuron. **C, D**, Summary plots of rebound delays versus the duration of hyperpolarization in mesoaccumbal neurons (light blue symbols) and nigrostriatal neurons (black symbols). Data are plotted as averages ± SEM.
**Figure 2-4 Effect of short duration hyperpolarizations in mesoaccumbal and nigrostriatal dopamine neurons.**

**A**, Example traces from a single mesoaccumbal dopamine neurons in which spontaneous firing was interrupted for 5 ms by a family of 20 pA current injection steps (left). The inset shows a zoomed in image of the 5 ms hyperpolarization. Center, Same example neuron to the left, depicting the 25 ms hyperpolarization, carried out in 5 pA steps. Right, Same example neuron depicting the 100 ms hyperpolarization done in 5 pA steps. All neurons were injected with a series of current injection steps for 5, 25, and 100 ms in the aforementioned current injection amplitudes, interleaved with the neuron's spontaneous firing. **B**, Example traces from a single mesoaccumbal dopamine neuron in which spontaneous firing was interrupted by separate 100 ms current injections. Top trace, A −35 pA injection hyperpolarized the membrane potential to a baseline voltage of −55 mV, resulting in an ISI₁ of 512.24 ms compared to an ISI₀ of 330.4 ms. Bottom trace, A −55 pA injection hyperpolarized the membrane potential to a baseline voltage of −65 mV, resulting in an ISI₁ of 668.94 ms compared to an ISI₀ of 320.66 ms. **C**, Same as in A for an example nigrostriatal dopamine neuron. Spontaneous firing was interrupted by a −125 pA (top) and a −300 pA (bottom) current injection, which hyperpolarized the cell to a baseline voltage of −56 mV and −65 mV. In the top trace, ISI₁ = 647.88 ms and ISI₀ = 540.06 ms. In the bottom trace, ISI₁ = 663.12 ms and ISI₀ = 457.70 ms. **D–F**, Voltage dependence of the ISI₁/ISI₀ ratio in response to 5 ms (D), 25 ms (E), and 100 ms (F) hyperpolarizations in mesoaccumbal (light blue symbols) and nigrostriatal (black symbols) subpopulations. Asterisks indicate that mesoaccumbal and nigrostriatal cells display significant differences in delay responses to 100 ms hyperpolarizations starting at baseline values of −55 mV. **G–I**, Plots of the average calculated resistance versus the current injection amplitude across all neurons within the mesoaccumbal (light blue symbols) and nigrostriatal (black symbols) subpopulations for 5 ms (G), 25 ms (H), and 100 ms (I) hyperpolarizations.
AmmTX3, a specific blocker of Kv4 subunits, abolishes rebound pauses of all durations in mesoaccumbal and nigrostriatal dopamine neurons.

**A**, Example of an averaged interspike interval from a spontaneously active mesoaccumbal dopamine neuron. The black line indicates linear fit to slope. **B**, Rebound delay from the same neuron. **C, D**, Plot of the relationship between the slope of the interspike voltage trajectory and the rebound delay. **E**, Example traces from a mesoaccumbal neuron demonstrating effect of 1M AmmTX3 on interspike voltage trajectory during pacemaking. The interspike interval slope increased from 23.66 to 109.1 mV/s in the presence of 1M AmmTX3. **F**, Effect of AmmTX3 on interspike voltage trajectory of a nigrostriatal neuron. The interspike voltage increased from 34.5 to 94.47 mV/s in AmmTX3. **G, H**, Traces demonstrating effect of AmmTX3 in reducing rebound pause in mesoaccumbal (E) and nigrostriatal (F) dopamine neurons. **I, J**, Averaged time course of normalized rebound delays under control conditions and following application of 1M AmmTX3 in mesoaccumbal (orange symbols; I) and nigrostriatal dopamine neurons (black symbols; J). Data are shown as averages ± SEM.
Figure 2.6 Pauses evoked by inhibitory synaptic stimulation and GABA uncaging reduced by AmmTX3 block of A-type potassium currents.

A, Effect of AmmTX3 on synaptically evoked pauses. Cell-attached recording of a pause in a VTA neuron evoked by 50 Hz stimulation for 300 ms under control conditions (black trace), in AmmTX3 (red trace), and in the presence of picrotoxin and CGP55845 (blue trace). Spikes are cropped. B, Averaged time course of inhibitory pause under control conditions and following bath application of AmmTX3 for 6 VTA neurons. C, Effect of AmmTX3 on inhibitory pauses evoked by RuBi-GABA uncaging. Cell-attached recording of a pause under control conditions (black trace) in AmmTX3 (red trace). Spikes are cropped. D, Summary plot of the effect of AmmTX3 block on uncaging-evoked pause in six VTA neurons.
Figure 2-7 Biophysical properties of A-type potassium currents midbrain dopamine neurons.

A, B, Voltage protocol (top) and example traces (bottom) of A-type potassium currents in mesoaccumbal (light blue traces; A) and nigrostriatal (black traces; B) dopamine neurons evoked by a family of voltage steps to −40 mV stepping from a range of voltages between −120 and −50 mV. C, Comparison of inactivation kinetics of A-type potassium currents evoked by steps from −90 to −40 mV. Inactivation time constants were determined from fits to single exponential functions. D, Summary of inactivation voltage dependence of half inactivation in mesoaccumbal (light blue symbols) and nigrostriatal (black symbols) dopamine neurons. E, Summary of inactivation time constant measured from steps to −40 mV in mesoaccumbal and nigrostriatal dopamine neurons. ***, P < 0.05.
Figure 2-8 Recovery from inactivation of A-type potassium currents in mesoaccumbal and mesostriatal dopamine neurons.

A, Voltage protocol (top) and example traces (bottom) testing recovery from inactivation following a 250 ms conditioning step from −70 to −40 mV. Recovery was tested over a range of intervals including 1–2000 ms. B, Plot of fraction of recovery for data shown in A. Current from test pulses were normalized to current evoked by conditioning pulse. Fit to a single exponential function is shown in red. C, Summary of recovery time constants for A-type currents recorded in mesoaccumbal and nigrostriatal neurons.
**Figure 2-9 Biophysical properties of H-current in mesoaccumbal and nigrostriatal subpopulations.**

*A, B,* Voltage protocols (top) and representative family of hyperpolarization-activated currents (IH; bottom) evoked by a range of steps from −120 to −80 mV, recorded in a mesoaccumbal (*A*) and nigrostriatal (*B*) dopamine neurons. *C,* Summary current–voltage relationship plots for H currents recorded from mesoaccumbal (light blue) and nigrostriatal (black) dopamine subpopulations. *D,* Summary of time constant of activation for H currents measured in mesoaccumbal and nigrostriatal dopamine subpopulations. Time constant of activation values were obtained by fitting an exponential to the raw current traces of H currents. *E, F,* Example traces displaying depolarizing sag potentials in response to hyperpolarizing current injections in mesoaccumbal (*E*) and nigrostriatal (*F*) neurons. Sag potentials were measured as the difference between the peak minimum and baseline voltages. *G,* Summary plot of sag amplitudes measured in mesoaccumbal and nigrostriatal dopamine neurons. ***, *P* < 0.05.
Figure 2-10 Correlation of inactivation kinetics of $I_A$ and the amplitude of $I_H$ with rebound properties in mesoaccumbal and nigrostriatal dopamine neurons. 

A, Left, Example of rebound delay in mesoaccumbal (MA) dopamine neuron. The linear fit to determine the rebound slope is shown in red. Right, Voltage-clamp-recorded $I_A$ (top) and $I_H$ (bottom) currents from same neuron shown in A. A-type potassium currents were elicited by a step to $-40$ mV (top), and $I_H$ currents were measured from a step to $-120$ mV. B, Plot of rebound slope versus $I_A$ inactivation time constant. Data from mesoaccumbal neurons are shown with light blue open symbols, and those from nigrostriatal (NS) neurons are shown with black open symbols. The linear fit to the data is shown in red along with Pr values. Light blue and black closed symbols are average ± SEM. C, Plot of rebound slope versus $I_H$ current amplitude. D, Plot of $I_A$ amplitude versus $I_H$ amplitude. E, Plot of rebound slope versus $I_A$ current amplitude. Note that the linear fit is not shown due to lack of correlation (Pr = −0.13).
Model DA Neuron

A

-90 mV

-30 mV

-70 mV

500 pA

25 ms

50 ms

100 ms

150 ms

200 ms

B

Rebound Delay (ms)

1200

800

400

Baseline $V_M$ (mV)

-80

-40

C

E

$I_A$, $\tau = 200$ ms

1X $I_H$

2X $I_H$

6X $I_H$

0 pA

500 ms

D

F

$V_M = -70$ mV

1X $I_H$

2X $I_H$

6X $I_H$

Rebound Delay (ms)

1200

800

400

$I_A$ Inactivation $\tau$ (ms)

0

50

100

150

200

G

$2X I_H$ 1X T-Type

$2X I_H$ 10X T-Type

$V_M \sim -70$ mV

500 ms
Figure 2-11 Computational model testing the relative contribution of $I_{A}$, $I_{h}$, and $I_{T}$ to the rebound delay and GABA$_{A}$-evoked pauses in firing.

A, Traces of simulated A-type potassium currents covering the experimentally observed range of inactivation time constants from 25 to 200 ms. $I_{A}$ currents were elicited by steps from −90 to −30 mV. Important to note is that the model conductances were adjusted to offset changes in peak current amplitudes resulting from alteration of decay time constants. B, Rebound delays increase in duration in models with slower $I_{A}$ inactivation kinetics. C, Voltage dependence of the rebound delay plotted in a model for $I_{A}$ time constant of inactivation ranging from 25 to 200 ms. Note the increase in the rebound delay with increasing voltage and with increase in the inactivation tau of $I_{A}$. D, GABA$_{A}$ evoked pauses increase in duration in models with slower $I_{A}$ inactivation kinetics. Simulated synaptic input was delivered at 50 Hz for 300 ms. E, Comparison voltage sags in three model neurons that range in relative peak conductance values of $I_{h}$ from one to six times peak, using an $I_{A}$ tau value of 200 ms. F, Plot of inactivation time constant of $I_{A}$ versus rebound delays, measured from a baseline voltage of −70 mV, in models with amplitudes of $I_{h}$ ranging from one to six times the peak. G, Comparison of rebound delay in two model neurons where T-type calcium is present at our control permeability value and at 10 times that value, with 200 ms of $I_{A}$ inactivation tau. Note the decrease in the rebound delay with T-type calcium present. H, Plot of rebound delay versus inactivation tau of $I_{A}$ in the our model neuron where T-type calcium current is present at our control permeability value (light blue circles) and 10 times that value (black circles).
CHAPTER 3

DIFFERENCES IN SMALL- (SK) CALCIUM-ACTIVATED POTASSIUM CURRENTS AND Kv2 CURRENTS CONTRIBUTE TO THE HETEROGENEITY IN THE FIRING OF MESOCORTICAL, MESOACCUMBAL AND MESOSTRIATAL DOPAMINE NEURONS.

Rahilla A. Tarfa and Zayd M. Khaliq
3.1 ABSTRACT

Cortical projecting (mesocortical) dopamine neurons fire at higher frequencies compared to dopamine neurons that project to the nucleus accumbens (mesoaccumbal) and the dorsal striatum (mesostriatal/nigrostriatal). Despite this, the ionic mechanisms contributing to this difference remains unknown. Using retrograde labeling and in vitro patch clamp electrophysiology in juvenile transgenic mice, we investigated the contribution of supra-threshold potassium currents to high frequency firing in dopamine neuron subpopulations. Consistent with previous data, we found that mesocortical dopamine neurons fired at slightly higher spontaneous and maximal firing rates, alongside significantly narrower action potential widths, shorter spike peaks and faster rates of repolarization. We found that the difference in maximal firing frequencies of the mesocortical and mesoaccumbal dopamine subpopulations could not be accounted for by differences in D2R currents. Given the differences in action potential width among the dopamine neuron subpopulations, we tested for the contribution of calcium-dependent potassium currents, and found that SK currents contributed to limiting the high frequency firing and reduced the evoked after-hyperpolarization (eAHP) among the mesoaccumbal, and by extension the mesostratal dopamine neurons. Finally, we used action potential clamp to test for the contribution of $K_{\text{V}}$2 and $K_{\text{V}}$3 currents to high frequency firing. We found a larger contribution of $K_{\text{V}}$2 currents as compared to putative $K_{\text{V}}$3 currents to outward currents evoked by depolarization and spike evoked interspike interval currents during high frequency firing. Moreover, we found that the amplitude of the $K_{\text{V}}$2 interspike interval current during high frequency firing ramped up at a slower time constant in the mesocortical as compared to SNc dopamine neurons, and weakly correlated with the deactivation kinetics of the $K_{\text{V}}$2 tail currents. This depicts that $K_{\text{V}}$2 currents might play a role in the difference in high frequency firing among the dopamine neuron subpopulations. Our work sheds light on additional currents that are different among the dopamine neuron subpopulations and may have implications for how they integrate information.
3.2 INTRODUCTION

Midbrain dopamine neurons function in brain circuits mediating reward signaling, motivation, aversion and movement. The wide variation in the function of dopamine dependent circuits is reflected in the functional heterogeneity of the dopamine neurons and their axonal projections to different brain regions. Consistent with this idea, early in vivo work in anesthetized rodents in which dopamine neuron subpopulations were identified via antidromic stimulation, demonstrated a delineation in the firing rates of the cortical projecting (mesocortical) and striatal projecting (mesostriatal/nigrostriatal) dopamine neurons (Chiodo et al., 1984). Mesocortical dopamine neurons fired at rates three times higher than that of mesostriatal dopamine neurons. This observation has been confirmed by recent in vitro work that made use of modern retrograde labelling techniques in mice that have the marker for dopamine neurons (Lammel et al., 2008). They demonstrated that mesocortical dopamine neurons fire action potentials at substantially higher rates than those projecting to the nucleus accumbens (mesoaccumbal) and the mesostriatal dopamine neurons (Lammel et al., 2008). To date however, the precise ionic mechanisms that underlie the heterogeneity in high frequency firing among the projection specific dopamine neurons has remained largely unexplored.

Aside from the heterogeneity in the excitability of the dopamine neuron subpopulations, another distinguishing feature among these neurons is their ability to auto-regulate their activity via D2 auto-receptors (Groves et al., 1975). The activation of D2 receptors is linked to the inwardly rectifying GPCR potassium channel (GIRKs), which allows the thoroughfare of potassium currents that significantly slows and halts the firing of dopamine neurons (Lacey et al., 1987, 1988; Kim et al., 1995). Interestingly, among the dopamine neuron subpopulations, the mesocortical dopamine neurons are unique in that they have been demonstrated to lack D2 auto-receptors, eliminating any form of autoregulation of their activity, while mesoaccumbal and mesostriatal dopamine neurons possess these receptors (Bannon et al., 1983; Chiodo et al., 1984; White and Wang, 1984a; Gariano et al., 1989b; Gariano et al., 1989a). The insensitivity of
the prefrontal cortex projecting dopamine neurons to dopamine is consistent with the sustained release of dopamine observed in the prefrontal cortex in vivo and the high affinity of the post-synaptic D1 receptors (Mundorf et al., 2001; Tzschentke, 2001; Stefani and Moghaddam, 2006). Interestingly, the presence of the dopamine auto-receptors has been shown to correlate with basal firing rates and the extent of burst firing in dopamine neurons in vivo (Chiodo et al., 1984). As such, one proposed explanation for the limited firing frequencies of mesoaccumbal and mesostriatal dopamine neurons is that the effect of dopamine on somatodendritically expressed D2 receptors limits their firing range (White and Wang, 1984a; White, 1996). However, an alternative to this idea is that it is the differential presence of intrinsic ionic currents among the dopamine neuron subpopulations instead that supports the differences in their high frequency firing.

In general, dopamine neurons fire at much lower rates during high frequency firing, with a rate in the range of 10 to 20 Hz, as compared to other central neurons that can fire up to 200 Hz e.g. Cerebellar Purkinje, Subthalamic nucleus and Substantia Nigra Reticulata among others (Llinas and Sugimori, 1980; Nakanishi et al., 1987b, a; Do and Bean, 2003; Khaliq et al., 2003). However, some in vivo and in vitro work have observed dopamine neurons that fire at rates greater than 20 Hz (Chiodo et al., 1984; White and Wang, 1984a; Lammel et al., 2008; Margolis et al., 2008). A few studies have examined high threshold currents and their contribution to high frequency firing among dopamine neurons. Past work has demonstrated the presence of K\textsubscript{V}2 and K\textsubscript{V}3 currents among the SNc dopamine neurons (Ding et al., 2011b; Dufour et al., 2014; Kimm et al., 2015). BK and K\textsubscript{V}2 currents have been shown to be both active during pacemaking, playing a redundant role and more so K\textsubscript{V}2 limiting the rate of high frequency firing in slices and dissociated cells while BK currents enhance high frequency firing in dissociated cells (Kimm et al., 2015). Till date however, no study has dissected the underlying supra-threshold potassium currents that enable high frequency firing within the context of the dopamine neuron subpopulations.
In this study, we combine the use of retrograde labelling and patch-clamp electrophysiology in TH-GFP (TH - Tyrosine Hydroxylase) mice to isolate dopamine neuron subpopulations and investigate the role of SK, Kv2 and Kv3 currents in enabling high frequency firing. We find that differences in the D2 receptor mediated currents cannot account for the disparity in the high frequency firing rates of dopamine neurons. Instead, we find that SK currents limit high frequency firing among the mesoaccumbal dopamine neurons, and mediates an evoked after-hyperpolarization (eAHP) that is different among the dopamine neuron subpopulations. Interestingly, we found that Kv2 currents are a larger contributor to both large step depolarizations and spike evoked interspike interval currents, as compared to putative Kv3 currents, regardless of the dopamine neuron subpopulation. More importantly, we demonstrate a novel build-up of spike-evoked interspike interval currents that is faster among SNc dopamine neurons as compared to VTA and mesocortical dopamine neurons, and is mediated by Kv2 currents. In conclusion, our work highlights the first efforts at characterizing the contribution of high threshold potassium currents to the differences in excitability among the dopamine neuron subpopulations.
3.3 METHODS

**Animal Husbandry:** Transgenic male and female mice that ranged in age from post-natal day 14 to 23, in which the expression of the green fluorescent protein is driven by the promoter for tyrosine hydroxylase (TH-GFP mice) were used in all experiments involving retrogradely-labeled VTA and SNc dopamine neurons (Matsushita et al., 2002). All mice were maintained according to the guidelines set by the Animal Care and Use Committee (ACUC) for the National Institute of Neurological Disorders and Stroke (NINDS) and the National Institutes of Health (NIH).

**Stereotaxic Brain Injections:** A Stoelting stereotaxic instrument for small animals was used to microinject the retrograde labeler cholera-toxin subunit B conjugated to Alexa-555 (CTB-AF555) into the left and right hemispheres of TH-GFP mice at postnatal days 15 to 18. Mice were anesthetized using 1.5% isoflurane. Scalp was shaved, cleaned with betadine and saline, injected with lidocaine (1%) and incised. Phosphate buffer solution (PBS) was used to keep the skull moist throughout surgery. The coordinates (bregma, lateral and ventral) for the prefrontal cortex (±0.3, ±1.7, -2.4 and -2.6 (2 different depths), lateral shell of the nucleus accumbens (±1.6, ±1.5, -4.6) and dorsal striatum (±1.6, ±1.4, -2.5) were used. CTB-AF555 (< 1.0 µL) was injected using a Hamilton micro syringe. After surgery, the scalp wound was closed using vet bond glue and mice were placed in an aerated cage to recover before being placed back in cage with parents and littermates. Mice were used for electrophysiology after a minimum of 3 days.

**Slice Preparation:** Coronal midbrain slices of 300 µm thickness were prepared from TH-GFP using a Microslicer DTK-Zero1. Slices were cut in an ice-cold glycerol-based slicing solution which consisted of the following ingredients (mM): 250 glycerol, 2.5 KCl, 2 MgCl₂, 2 CaCl₂, 1.2 NaH₂PO₄, 10 HEPES, 21 NaHCO₃ and 5 glucose. Slices were incubated in a warm 33°C bath containing recording solution simultaneously bubbled with 95% O₂ / 5% CO₂ for 30 minutes and incubated at room temperature for another 30 minutes. Recording solution consisted of the
following (mM): 125 NaCl, 25 NaHCO$_3$, 1.25 NaH$_2$PO$_4$, 3.5 KCl, 10 glucose, 5 HEPES, 1 MgCl$_2$ and 2 CaCl$_2$.

**Patch Clamp Electrophysiology:** Midbrain slices containing the VTA and SNc were placed in a heated recording chamber that was continuously perfused with heated ACSF with temperatures ranging from 31-33°C. Candidate neurons were first visualized and located using an upright Olympus microscope BX50WI connected to a Hamamatsu camera. Neurons that fluoresced green using the GFP long-pass filter were identified as dopamine neurons, and neurons that fluoresced red were identified as neurons that project to the brain region that had been injected with CTB-AF555 (either Prefrontal Cortex, Nucleus Accumbens or Dorsal Striatum). Neuron were recorded only if they were positive for both red and green fluorescence, indicating that they were dopamine neurons that projected to the either the nucleus accumbens or dorsal striatum.

Neurons were recorded using borosilicate recording electrodes (VWR international) pulled using a flaming/brown micropipette puller (Sutter Instrument Company). Pipettes were filled with an internal recording solution that consisted of the following (mM): 135 KMeSO$_3$, 10 NaCl, 10 HEPES, 2 MgCl$_2$, 0.5 EGTA and 0.1 CaCl$_2$. Electrodes were wrapped with parafilm to reduce electrode capacitance. Brain slices were placed in a recording chamber that was constantly perfused with oxygenated recording solution. Slices were visualized with an upright microscope (Olympus) via an IR-DIC prism with 4x and 60x objectives. All data were collected using pClamp10 software (Axon Instruments), recorded on a Multiclamp 700B amplifier and digitized on a Digidata 700B. Data were filtered at 10 kHz and sampled at 20 kHz. Series resistance and whole cell capacitance were compensated using the whole cell and Rs compensation features and Rs monitored frequently. Cells with uncompensated series resistances of > 16 MΩ were immediately discarded. All experiments were performed at 31-33°C.
Spontaneous and Evoked Firing: To maximize the number of recordings displaying healthy firing activity, spontaneous firing was monitored for a period in cell-attached configuration. The membrane was then ruptured while recording in current clamp mode, which allowed for easy identification of firing rate changes due to damage during breakthrough. Initial spontaneous firing activity was obtained for 1-2 min during which the health of the neuron was monitored - if the spontaneous firing rate dramatically differed from the firing rate during the cell-attached configuration, the cell would be discarded. Bridge balance was often corrected using the automatic function of the Multiclamp amplifier. Neurons had been hyperpolarized with sufficient current injection to enable the membrane voltage to be at -70 mV, after which Frequency-intensity (FI) curves were obtained for each neuron by providing a series of depolarizing current injections (40 pA steps, 1 second duration) during their spontaneous activity. For experiments testing the contribution of D2 currents to evoked high frequency firing, Barium (100 nM) and Sulpiride (10 uM) were bath applied to block the G-protein inwardly rectifying potassium currents (GIRKs) and D2 auto-receptors, and these were within cell experiments. Experiments testing the contribution of the big- (BK) and small- (SK) conductance calcium activated potassium currents to high frequency firing involved the bath application of paxilline (1uM) and apamin (300 nM) respectively and were carried out as population experiments with the control group of neurons separate from the drug applied group of neurons.

Action potential clamp (voltage clamp): Previously recorded high frequency firing evoked from -70 mv in an example mesocortical and substantia nigra compacta (SNc) dopamine neuron were used as the voltage command in voltage clamp in order to record the corresponding currents. All neurons were compensated using the digitata amplifier circuitry. Cells with series resistance of up to 16 MΩ were included and compensated for using their estimated series resistance and capacitance at 70 – 80% compensation. For the control traces, 500 nM – 1 uM of TTX, 10 uM Paxilline and 300 nM Apamin were bath applied to block
sodium, BK and SK currents. In some cases, 0 mM Ca\(^{2+}\) was used instead to block calcium
independent currents for the control conditions (this was only done in the mesoaccumbal
dopamine neurons and is indicated in the figure). 100 nM - 200 nM of Guangitoxin (GTX) was
used to block K\(_V\)2 currents and 1 mM TEA was used to block K\(_V\)3 currents. Controls and drug
applications were carried out within the same cell.

**Histology and Confocal Imaging:** Slices with retrogradely labeled dopamine neurons were
placed in 4% paraformaldehyde for at least 24 hours and rinsed three times with PBS before
being mounted onto glass slides with mounting medium (Vectashield). Slices were imaged
using a Leica CW microscope with an inverted 10X objective for images of the whole tissue
depicting injection site and 63x for the individual neurons. Images were processed using
ImageJ (NIH).

**Data Analysis and Statistics:** All electrophysiological traces were analyzed using Igor
(Wavemetrics). To quantify the spontaneous firing properties, a train of healthy spikes (as
indicated by no change in the spike peak and trough) were chosen and spike statistics were
performed. Average spontaneous rate was measured as the average interspike interval during
a 2-3 second period of the spike train. The maximal firing rate taken as the rate immediately
preceding entry into depolarization block. The after-hyperpolarization that followed evoked high
frequency firing (eAHP) was measured as the difference between the minimum point of the
eAHP and the baseline voltage (before the evoked depolarization).

The junction potential of our methanesulfonate-based internal solution was measured
at -8.2 mV, however, the data presented were not corrected. All statistics were carried out
using Student’s t-test except where noted. Average values are reported as average ± S.E.M.
3.4 RESULTS

3.4.1 Higher firing rates among the Mesocortical dopamine subpopulation as compared to the Mesoaccumbal and Mesostriatal dopamine subpopulation.

To isolate the mesocortical, mesoaccumbal and nigrostriatal dopamine subpopulations according to their axonal projections, we injected the retrograde tracer Cholera toxin subunit B (CTB) conjugated to the Alexa fluorophore 555 (AF-555) into the known brain regions that these dopamine neurons target in transgenic TH-GFP mice. Injections into the prefrontal cortex resulted in sparse labelling of neurons in the VTA, with the majority located in the medial portion of the VTA, and little to no labeling in the lateral portions of the VTA and the SNc (data not shown). On the other hand, injections into the nucleus accumbens and dorsal striatum resulted in a higher density of labeled neurons. Most of the dual labeled neurons were in the VTA for the nucleus accumbens injected site, with some neurons found on the border of the VTA and SNc, and very few in the SNc (see Chapter 2; Figure 1A). Injections into the dorsal striatum always resulted with labeled neurons in the SNc, with some located on the border and little to none in the VTA (see Chapter 2; Figure 1B). Dual-labeled neurons from injections into the nucleus accumbens and dorsal striatum demonstrate the contiguous borders of the SNc and VTA, demonstrating the mixture of dopamine neuron subpopulations that exist here. This highlights the value of examining dopamine neurons in the context of the subpopulations to understand the underlying principles that contribute to their heterogeneity. Whole-cell recordings were only made from dual-labelled neurons of GFP and AF-555, which demonstrates that it was a dopamine neuron that projects to the injected brain region of interest.

To ascertain the degree of heterogeneity in the excitability of the dopamine neuron recordings, whole-cell patch clamp recordings of spontaneous and evoked firing rates were obtained in current clamp from mesocortical, mesoaccumbal and mesostriatal dopamine neurons. All neurons included in the spontaneous data set spontaneously fired upon
breakthrough, however, some mesocortical dopamine neurons were noted to be silent on breakthrough, but fired normally with injected current. These neurons were included in the evoked firing data set. In examining the spontaneous activity of the dopamine neurons, on average, neurons within the three subpopulations fired well within the expected range (1-5 Hz) for dopamine neurons in slices (MC: 3.73 ± 0.60 Hz, N = 24; MA: 2.82 ± 0.14 Hz, N = 89; MS: 2.43 ± 0.14, N = 72) (Figure 1A-C & G). Despite the small range in difference, the average firing rates were significantly different between the mesocortical and mesoaccumbal (p-value = 0.03), mesocortical and mesostriatal (p-value = 0.0026) but trending between mesoaccumbal and mesostriatal (p-value = 0.056) dopamine neurons (Figure 1G). We also examined the maximal firing rates of the dopamine neurons by hyperpolarizing spontaneously firing neurons with current injection until their membrane voltage was at ~70 mV, and injected a family of depolarizing current injections to obtain a frequency-intensity curve (Figure 1D-F). All three subpopulations displayed an overlap in firing rates for current injections less than 100 pA, however, beyond that point, the F-I curve became increasingly steep for the mesocortical dopamine neurons as compared to the mesoaccumbal and mesostriatal neurons (Figure 1H). We quantified the maximal firing rate, which is defined as the firing rate immediately prior to the neurons entering depolarization block. Mesocortical dopamine neurons fired with the highest maximal rate at: 22.22 ± 2.05 Hz (N = 35), followed by mesoaccumbal at: 14.89 ± 1.71 Hz (N = 24) and lastly mesostriatal firing at the lowest rates of: 10.7 ± 0.66 Hz (N = 11) (Figure 1I). Our work is consistent with past work that demonstrated that mesocortical dopamine neurons fire at higher maximal rates (on average 15 Hz) in response to a ramp of current injection (Lammel et al., 2008). However, our work differs in that we did not find any dopamine neurons, even among the mesocortical dopamine neurons, that fired above 10 Hz during spontaneous activity (range: 0.5 – 9.1 Hz, N = 24). Altogether, consistent with past reports (Chiodo et al., 1984; White and Wang, 1984b; Lammel et al., 2008), our work demonstrates the increased excitability that is present among mesocortical dopamine neurons as compared to the
mesoaccumbal and mesostriatal dopamine neurons.

Given the differences in the firing frequencies of the dopamine neurons subpopulations, we further examined the individual spike features of the dopamine neurons. We measured and plotted the average half-width (defined as the width of the action potential at the half-way point between the trough and peak of the action potential), spike peak, spike trough, upstroke (rate of depolarization) and downstroke (rate of repolarization). We found that mesocortical dopamine and mesoaccumbal dopamine neurons displayed narrower action potential widths as compared to mesostriatal dopamine neurons (MC: 1.48 ± 0.078 Hz (N = 24), MA: 1.53 ± 0.036 Hz (N = 89) and MS: 1.74 ± 0.039 Hz (N = 72)) (Figure 2A-C & G). Despite the small difference in values, mesocortical (MC vs MS, p-value = 0.0018) and mesoaccumbal (MA vs MS, p-value = 9.93E-05) dopamine neurons were significantly narrower than mesostriatal dopamine neurons. Given the difference in the half-width, we also examined the action potential’s rate of repolarization (downstroke), and observed that it was fastest among the mesocortical and mesoaccumbal as compared to the mesostriatal dopamine neurons (MC: -58.59 ± 1.82 mV/ms (N = 24), MA: -60.42 ± 3.69 mV/ms (N = 89) and MS: -50.10 ± 1.67 mV/ms (N = 72)) (Figure 2D-F, H). Along these lines, we examined the correlation between the half-width of the action potentials and the rate of repolarization and as expected saw a strongly positive correlation in all three subpopulations (Figure 2I). The troughs for mesocortical dopamine neurons were the most depolarized as compared to mesoaccumbal and mesostriatal dopamine neurons (MC: -52.97 ± 1.46 mV (N = 24), MA: -56.18 ± 0.60 mV (N = 89) and MS: -55.53 ± 0.68 mV (N = 72)) (Figure 2J). Mesocortical dopamine neurons also displayed the shortest peak height at MC: 18.9 ± 1.65 mV (N = 24), while mesoaccumbal and mesostriatal dopamine neurons displayed the tallest peaks (MA: 26.30 ± 1.01 mV (N = 89) and MS: 26.99 ± 1.15 mV (N = 72)) (Figure 2A-C & L). Lastly, the rate of depolarization (upstroke) was highest in the mesoaccumbal as compared to the mesocortical and mesostriatal dopamine neurons (MC: 105.20 ± 9.38 mV/ms (N = 24), MA: 133.15 ± 5.58 mV/ms (N = 89) and MS:
114.18 ± 5.07 mV/ms (N = 72)) (Figure 2D-F, K). Together our work demonstrates that mesocortical dopamine neurons possess the narrowest spikes, most hyperpolarized peaks, most depolarized troughs, slowest rate of depolarization and fastest speed of repolarization as compared to the mesoaccumbal and mesostriatal dopamine neurons. Overall, the firing data obtained from the dopamine neuron subpopulations raises the possibility that the ionic currents that underlie firing could be heterogeneous, depending on the dopamine neuron subgroup being examined.

3.4.2 Differences in the D2 auto-receptor currents among the dopamine neuron subpopulations do not limit high frequency firing.

From previous work on the dopamine neuron subpopulations, it can be postulated that the impulse regulating ability present in mesoaccumbal and mesostriatal dopamine neurons enables their activity to remain at lower rates as compared to the mesocortical dopamine neurons which demonstrate sustained release of dopamine (White and Wang, 1984a; White, 1996; Tzschentke, 2001; Lammel et al., 2008). We sought to test this hypothesis that differences in the D2-currents could underlie the differences in the firing rates of the dopamine neuron subpopulations. We examined the firing of mesocortical and mesoaccumbal dopamine neurons evoked from a membrane voltage of ~70 mV in the presence and absence of barium (100 uM) to block the GIRK channels and sulpiride (10 uM) to occlude the D2-receptors, within the same neurons. Figure 3 demonstrates examples of maximal firing in a mesocortical and mesoaccumbal dopamine neuron in control conditions (Figure 3A and B) and minutes later in the presence of barium and sulpiride (Figure 3C and D), within the same neuron. We examined the maximal firing rates in the neurons and quantified it as the firing rate right before depolarization block. In the mesocortical dopamine neurons, we found a maximal firing rate of 45.85 ± 8.93 Hz, which remained similar in the presence of barium and sulpiride at 41.65 ± 7.49 Hz (N = 5; p-value = 0.73). We performed the same experiments in mesoaccumbal dopamine neurons and found a maximal firing rate of 27.6 ± 3.0 Hz, which was also similar in
the presence of barium and sulpiride at 25.82 ± 1.8 Hz (N = 6; p-value = 0.62) (Figure 3E and F). We further examined the changes in firing frequency in response to different amplitudes of current injections in both the mesocortical and mesoaccumbal dopamine neurons and obtained a frequency-intensity plot both in groups. We found that in both control and test conditions (barium and sulpiride) the frequency of the mesocortical dopamine neurons increased with current injection, demonstrating the same steepness seen in Figure 3. The F-I curve was completely overlapping both in the presence and absence of Barium and Sulpiride in the mesocortical neurons (Figure 3G). We observed similar results in the mesoaccumbal dopamine neurons, wherein the firing rates were the same in control conditions as in the presence of Barium and Sulpiride (Figure 3H). Finally, we measured the input resistance both before and after the application of barium and sulpiride and noted no significant change in the input resistance in both subpopulations (R_in avg ± SEM: MC pre-drug: 240.21 ± 73.07 MΩ, MC post-drug: 332 ± 161.82 MΩ, p-value = 0.32; MA pre-drug: 148.73 ± 33.27 MΩ, MA post-drug: 167.02 ± 34.78 MΩ, p-value = 0.71). As such, under our set of conditions, our data demonstrates that the difference in D2 receptor mediated currents do not underlie the heterogeneity in the high frequency firing rates observed among mesocortical and mesoaccumbal dopamine neurons. Therefore, together with the observed differences in the spike features of the dopamine neurons subpopulations in Figure 2, our data shows that intrinsic ionic mechanisms rather than extrinsic factors underlie the spiking differences among the dopamine neuron subpopulations.

3.4.3 Distinct difference in a high frequency evoked after-hyperpolarization (eAHP) is mediated by the small-conductance calcium-activated potassium (SK) current.

During our experiments quantifying the evoked high frequency firing among the dopamine neuron subpopulations, we noticed a distinct after-hyperpolarization that subsequently followed high frequency firing when the neuron’s membrane was hyperpolarized
back to the holding voltage of ~70 mV. However, this distinct after-hyperpolarization was unevenly distributed among the neurons. As this is an AHP that follows high frequency firing evoked by depolarizing current injections, we termed it the ‘evoked after-hyperpolarization’ (eAHP). We quantified and calculated the eAHP as the difference between the baseline voltage in the neuron (before high frequency firing) and the minimal peak value following high frequency firing. We also sorted the eAHP according to the dopamine neuron subpopulations. Figure 4A-C demonstrates examples from each dopamine neuron subpopulation wherein the eAHP is labeled. The example mesocortical dopamine neuron had the smallest eAHP amplitude of -2.34 mV, followed by the mesoaccumbal dopamine neuron at -13.06 mV and the largest amplitude in the mesostriatal dopamine neuron at -18.49 mV. We then quantified the average eAHP amplitudes within each dopamine neuron subpopulation and plotted them against the firing frequency of the neuron during the evoked membrane depolarization and observed the same trend (MC: -7.46 ± 0.66 mV at 14.74 Hz; MA: -12.67 ± 0.60 mV at 14.0 Hz and MS: -20.39 ± 0.57 mV at 14.10 Hz with MA vs MC, p-value = 0.00044; MA vs MS, p-value = 0.00016 and MC vs MS, p-value = 2.71E-10 (Figure 4D)). While this AHP is distinct from the typical definition of an AHP that occurs after an action potential, the deep hyperpolarization of the trough that followed high frequency firing and its large amplitudes selectively displayed in the mesoaccumbal and mesostriatal dopamine neurons are closely characteristic of a calcium mediated potassium current. As such, we sought to uncover the major ionic current(s) underlying the eAHP, and hypothesized that it could be due to the disproportionate presence of calcium activated potassium channels among the mesoaccumbal and mesostriatal dopamine neurons. Therefore, we tested for the contribution of the big conductance (BK) and small conductance (SK) calcium activated potassium currents to the amplitude of the eAHP. First, we tested the effect of the specific blockers for BK and SK, paxilline (10 µM) and apamin (300 nM), on the amplitude of the eAHP in a population of mesoaccumbal dopamine neurons. In the presence of apamin and paxilline, we found that the amplitude of the eAHP was significantly
decreased at higher frequencies as compared to the population of control mesoaccumbal neurons: (Control MA vs apamin + paxilline MA: MA: -15.67 ± 1.47 mV at 24.08 Hz vs 8.60 ± 0.38 mV at 24.99 Hz; p-value = 0.029) (Figure 5A, B and D). This highlighted the possible contribution of calcium-activated currents to the eAHP, however to further dissect which current played a larger role in shaping the eAHP, we carried out separate experiments in apamin. We tested for the contribution of SK in the presence of apamin, and we observed a significant reduction in the eAHP (Figure 5A, C and D) (Control MA vs apamin + paxilline MA: -12.67 ± 0.60 mV at 14.0 Hz vs -9.6 ± 0.72 mV at 24.39 Hz; p-value = 0.0021).

In addition to the amplitudes of the eAHP, we also observed an increase in the evoked firing frequency in response to all injected current amplitudes in the apamin alone and apamin + paxilline conditions as compared to the control condition among the mesoaccumbal dopamine neurons (firing rates at 400 pA current injection: MA control = 14.05 ± 1.073 Hz, MA apamin + paxilline = 24.24 ± 4.69, and MA apamin = 20.75 ± 3.30) (Figure 5E). Lastly, given recent work that has demonstrated the role of BK currents in contributing to high frequency firing in SNc dopamine neurons, we examined the contribution of BK currents to high frequency firing among the mesoaccumbal dopamine neurons and observed no significant contribution (firing rates at 400 pA current injection: MA control = 14.05 ± 1.073 Hz and MA paxilline = 17.15 ± 2.47). Altogether, our work demonstrates the selective presence of a SK-mediated eAHP in mesoaccumbal and by extension, the mesostriatal dopamine neurons, and shows that SK currents are a major contributor to limiting high frequency firing in these dopamine neurons.

3.4.4 The contribution of high threshold delay rectifier potassium (K⁺) currents to large step depolarizations among dopamine neuron subpopulations.

The differences in the rates of repolarization (downstroke) (Figure 2) observed among the dopamine neuron subpopulations point to the possibility of a heterogeneity in the repolarizing currents, which tend to be high-threshold potassium currents. Previous work in
dopamine neurons has established the role of BK and Kv2 currents in shaping the repolarization phase of the action potential (Kimm et al., 2015). Similarly, Kv3 currents have been known to play a primary role in enabling fast rates of repolarization among central nervous system neurons (Rudy and McBain, 2001; Ding et al., 2011b). However, differences in these currents have yet to be examined among the dopamine neuron subpopulations. We therefore hypothesized that differences in the Kv2 and Kv3 currents could underlie the heterogeneity in the high frequency firing rates among the dopamine neuron subpopulations. Given the somatodendritic presence of the Kv2 and Kv3 channels in the soma and mostly proximal dendrites (Guan et al., 2007; Dufour et al., 2014), we examined for the presence and contribution of Kv2 and Kv3 currents to large step depolarizations injected into the soma in intact dopamine neurons within the midbrain slice. Both currents were examined by stepping the membrane voltage from -40 mV to 0 mV and the contribution of Kv2 and Kv3 currents were examined in mesocortical, mesoaccumbal and SNc dopamine neurons. In all mesoaccumbal dopamine neurons, experiments were carried out in 0 mM calcium while mesocortical and SNc experiments were carried out in the presence of apamin and paxilline to block the calcium dependent potassium currents. Figure 6A-C demonstrates examples of the currents in the absence (black traces) and presence of the Kv2 channel blocker (dark green traces) and Guangxitoxin (GTX, 200 nM), with the blocked GTX sensitive currents underneath (light green traces). In the example mesocortical neuron, 54.18 % of the control current is GTX sensitive, as compared to 30.49 % in the mesoaccumbal and 41.75 % in the SNc dopamine neuron (Figure 6A-C). Similarly, on average, we found that 38.3 ± 4.97 % was blocked by GTX in mesocortical dopamine neurons (N = 5) as compared to 47.03 ± 5.25 % in the mesoaccumbal (N = 5) and 49.17 ± 6.14 % SNc dopamine neurons (N = 9) (Figure 6G-I). In all neurons, we also washed on
TEA* (1 mM) to block Kv3 currents. Figure 6D-F shows examples of currents recorded in the absence (black traces) and presence of TEA (dark brown traces), in mesocortical, mesoaccumbal and SNc dopamine neurons. TEA sensitive currents are depicted underneath (light brown traces). In the examples, 46.72 % of the leftover current in GTX was further blocked by TEA in the mesocortical dopamine neuron as compared to 52.01 % and 74.97 % in mesoaccumbal and SNc dopamine neurons respectively. On average, we found that 26.74 ± 2.27 % of the control current was blocked by TEA in mesocortical dopamine neurons (N = 5) as compared to 21.74 ± 2.66 % in the mesoaccumbal (N = 5) and 29.69 ± 4.71 % in SNc dopamine neurons (N = 9) (Figure 6G-I). We found that the contribution of the GTX sensitive currents as compared to the TEA sensitive currents was larger and significant among the mesoaccumbal (p-value = 0.0055) and trending in the SNc (p-value = 0.048) dopamine neurons as compared to insignificance among the mesocortical dopamine neurons (p-value = 0.068). Together, our data demonstrates the large contributions of Kv2 and Kv3 currents to outward currents evoked from step depolarizations, with Kv2 currents contributing almost half of the leftover calcium independent outward current (after paxilline and apamin or in 0 mM Ca\(^{2+}\)) as compared to Kv3 currents which contribute about a quarter of the leftover calcium independent outward current.

3.4.5 Examining the contribution of high threshold delayed rectifier potassium (K\(^+\)) currents to spike evoked interspike interval currents in dopamine neurons.

Given the abundant evidence and contribution of Kv2 and Kv3 currents to outward currents evoked in response to large step depolarizations, we wanted to examine the contribution of these two currents to high frequency firing under more physiological conditions. As such, using action potential clamp, we directly examined the ionic currents underlying high frequency firing in two of the dopamine neuron subpopulations that demonstrated the highest

* Note that TEA was washed on in the presence of TTX and 0 mM Ca\(^{2+}\) or TTX, paxilline and apamin, as such, we can be reasonably assured that the TEA sensitive currents are putative Kv3 currents.
contrast in firing rates – mesocortical and SNc dopamine neurons. Previously recorded evoked high frequency firing from a mesocortical (29.6 Hz) and SNc neuron (24.2 Hz) were played back to the dopamine neurons in voltage clamp, to record the spike evoked currents. Contributions from the K\textsubscript{V}2 and K\textsubscript{V}3 currents were examined by washing on 200 nM of Guangxitoxin (GTX) and 1 mM of TEA in the presence of TTX, Paxilline and Apamin to block sodium, BK and SK currents respectively. Calculations were made from the spike evoked interspike interval currents to minimize the contribution from the voltage error that would result from the large action potential voltage changes.

First, we measured the currents in mesocortical and SNc dopamine neurons in response to a high frequency firing waveform recorded from a mesocortical dopamine neuron (we call this the mesocortical waveform in the graph). Figure 7A and B shows the high frequency firing that served as the command voltage and the corresponding currents (black = control condition, green = GTX condition and dark yellow = TEA condition) that flowed in response to the command voltage in an example mesocortical (Figure 7A) and SNc dopamine neuron (Figure 7B). As the effect of the block of GTX and TEA was more dramatic towards the end of the firing train as compared to the beginning, we quantified the amplitude of the last two interspike intervals (ISIs) in the train as an average and compared it in the control (black trace), GTX (dark green trace) and TEA (dark yellow trace) conditions (Figure 7C and D). Among the mesocortical dopamine neurons, on average, we found that the control ISI was 657.62 ± 125.34 pA (N = 5), which was reduced to 319.66 ± 62.41 pA (48.6%) in the presence of GTX, and in TEA, it was further reduced to 164.16 ± 40.51 pA (24.96 % of the leftover current in GTX) (Figure 8C). We also examined the contribution of GTX and TEA sensitive currents to the spike-evoked interspike interval currents in SNc neurons (Figure 8B, D and F). In control conditions, we found that the average of the last two ISIs was 854.76 ± 147.56 pA, which reduced to 45.35 % in GTX with a value of 387.62 ± 112.92 pA and was further reduced to 22.04 % of the control currents with a value of 188.41 ± 66.28 pA in TEA (Figure 8D). On average, the GTX sensitive currents
in the mesocortical dopamine neurons consisted of 51.32 ± 5.12 % (N = 5) of the control interspike interval currents, which was significantly greater than the contribution of the TEA sensitive currents quantified at 25.09 ± 3.99 % (p = 0.00375) (Figure 8E). Among SNc dopamine neurons, the contribution of GTX sensitive currents was similar to the levels seen in mesocortical dopamine neurons at 56.23 ± 6.18 % (N = 11) and were larger than the TEA sensitive currents at 23.88 ± 3.72 % (p = 0.00023) (Figure 8F).

Next, we measured the interspike interval currents in response to high frequency firing recorded from a SNc dopamine neuron and played it back in mesocortical and SNc dopamine neurons. Figure 8A and B demonstrates examples of the command voltage that was recorded in a SNc dopamine neuron in an example mesocortical (Figure 8A, top) and example SNc (Figure 8B, bottom) dopamine neuron. On average, among the mesocortical dopamine neurons, we found that the control ISI was 283.24 ± 50.37 pA (N = 5), which was reduced to 136.37 ± 21.02 pA (48.15%) in the presence of GTX, and in TEA, it was further reduced to 95.71 ± 18.64 pA (33.79 % of the leftover current in GTX) (Figure 8C). Among the SNc dopamine neurons, the control ISI was 369.37 ± 59.92 pA (N = 5), which was reduced to 170.75 ± 44.95 pA (46.22%) in the presence of GTX, and in TEA, it was further reduced to 93.25 ± 36.85 pA (25.25 % of the leftover current in GTX) (Figure 8D). Furthermore, we found that GTX-sensitive currents were a larger contributor to the spike-evoked interspike interval currents, as they made up 50.33 ± 3.15 % of the control current in mesocortical dopamine neurons and 54.57 ± 7.15 % of the control current in SNc dopamine neurons (Figure 8E). On the other hand, TEA-sensitive currents made up 30.85 ± 4.53 % of the leftover current in mesocortical dopamine neurons, and 23.24 ± 5.28 % in SNc dopamine neurons (Figure 8F). Altogether, our data demonstrates the overwhelming contribution of Kv2 currents, as compared to Kv3 currents, to the interspike interval currents evoked during high frequency firing in both mesocortical and SNc dopamine neurons. Notably, we did not find that the proportional contribution of Kv2 and Kv3 currents were different in the mesocortical as compared to the SNc dopamine neurons.
3.4.6 Kv2 currents accumulate differentially during high frequency firing in mesocortical and SNc dopamine neurons.

In examining the spike evoked Kv2 and Kv3 interspike interval currents during high frequency firing via action potential clamp, we observed a distinct increase in the amplitude of the interspike interval currents evoked during the high frequency train which we refer to as the ‘build up current’ or ‘BUC’ (Figure 8A bottom). Interestingly, this BUC seemed to dissipate in the presence of GTX and seemed to be more obvious in currents that were recorded in response to the high frequency train that was previously recorded from a mesocortical dopamine neuron that served as the command voltage (the mesocortical waveform) (compare Figures 8A vs 8B bottom). Figure 9 shows an example of the mesocortical high frequency train that served as the command voltage in a mesocortical (Figure 9A), unlabeled VTA (Figure 9B) and unlabeled SNc (Figure 9C) dopamine neuron. The light green trace underneath represents the GTX sensitive trace that demonstrates the time dependent buildup in the amplitude of the interspike interval (BUC) observed in all three sub-types of the dopamine neurons examined (Figure 9 A-C). We fitted the entire duration of the BUC, which showed a build-up time constant of 379.18 ms in the example mesocortical dopamine neuron, 336.76 ms in the example VTA dopamine neuron and 231.31 ms in the example SNc dopamine neuron (Figure 9A-C, dotted lines = fit). On average, mesocortical dopamine neurons had a BUC time constant of 378.23 ± 42.06 ms (N = 5), which was similar to the BUC time constant observed in unlabeled VTA dopamine neurons at 403.85 ± 54.67 ms (N = 13) (p-value = 0.72) (Figure 9J). However, when compared to the BUC time constant in SNc dopamine neurons, the BUC time constant which was 219.37 ± 38.73 ms (N = 11) was significantly different from mesocortical (p-value = 0.011) and unlabeled VTA (p-value = 0.013) dopamine neurons (Figure 9J). Together, our data demonstrates the presence of a cumulative build-up of GTX sensitive interspike interval currents during high frequency firing that is present and slower in mesocortical and unlabeled VTA dopamine neurons, as compared to SNc dopamine neurons.
While we observed no difference in the proportion of the $K_v2$ currents in the different subpopulations, the observance of a difference in the cumulative build-up current (BUC) raises the possibility that the build-up current could be due to a difference in the deactivation kinetics of the $K_v2$ current. As such, we hypothesized that the slow deactivation kinetics of the $K_v2$ current could result in an accumulation of currents during the interspike interval. We tested this idea by measuring the tail currents to obtain the deactivation kinetics of the $K_v2$ currents and compared it to the kinetics of the build-up currents during high frequency firing within the same dopamine neuron. First, we depolarized the membrane from $-40$ mV to $0$ mV to elicit large outward currents in both mesocortical and mesoaccumbal dopamine neurons. To obtain tail currents and measure the deactivation kinetics, we stepped down to $-50$ mV. Slices were bathed in TTX, paxilline and apamin, and then GTX to obtain the GTX-sensitive current. The tail current was fitted with an exponential to obtain the kinetics. Figure 9D-F demonstrates examples of the GTX-sensitive tail currents recorded in mesocortical, unlabeled VTA and SNc dopamine neurons. On average, we found that the tail current deactivated with a time constant of $39.31 \pm 2.71$ ms (N = 5) among mesocortical dopamine neurons as compared to $46.62 \pm 9.69$ ms (N = 9) in the SNc dopamine neurons (p-value = 0.59) and $45.95 \pm 7.92$ ms (N = 13) (p-value = 0.62) in the VTA dopamine neurons (Figure 9K). To further examine the relationship between the deactivation kinetics of the $K_v2$ currents, we quantified the correlation between the decay time constant of the GTX sensitive tail current and the build-up current within the same neuron. We found a weakly negative correlation between the BUC time constant and the decay time constant of the tail current ($P_r = -0.366$ and p-value = 0.060) (Figure 9L). As such, while our data demonstrates no difference in the deactivation kinetics of the $K_v2$ tail currents in mesocortical and SNc dopamine neurons, we found that the decay kinetics of the $K_v2$ currents might play a role, albeit weak, in contributing to the time it takes for spike-evoked $K_v2$ currents to accumulate during high frequency firing.
3.5 DISCUSSION

A few studies have highlighted the heterogeneity in the excitability properties of dopamine neuron subpopulations, yet little remains known about the ionic currents that mediate these differences. Most studies have focused on the ionic currents that underlie pacemaking with only a few examining the currents that underlie said high frequency firing. Here, we present evidence that differences in the calcium-activated potassium current – SK, and the voltage-gated potassium current, Kv2, contribute to the differences in excitability observed among dopamine neuron subpopulations.

3.5.1 Differences in D2-receptor mediated currents do not underlie high frequency firing.

We investigated the hypothesis that differences in the dopamine auto-receptor (D2) can account for the observed differences in the firing frequencies of dopamine neuron subpopulations in both our work and previous literature (Chiodo et al., 1984; White and Wang, 1984a; Lammel et al., 2008; Margolis et al., 2008). We found that the presence of both D2 receptor antagonists and blockers of GIRK currents did not substantially affect the evoked firing rates of the mesocortical and mesoaccumbal dopamine neurons. As such, under our set of conditions, our work provides evidence that the differences in evoked high frequency firing among these two subpopulations cannot be attributed to the extrinsic influence of dopamine on their D2-autoreceptors. While these experiments were not carried out in mesostriatal dopamine neurons, no differences in dopaminergic self-regulation have been reported between mesoaccumbal and mesostriatal dopamine neurons (Clark and Chiodo, 1988; Lacey et al., 1988; White, 1996). Previous work has demonstrated that spontaneous and evoked IPSCS that are D2-receptor mediated, activate GIRKS (Beckstead et al., 2004; Gantz et al., 2013). As such, under our set of conditions, we assume that the somatodendritic release of dopamine is occurring, although we do not explicitly test for this. Future experiments should directly test the
somatodendritic release of dopamine in response to increased current evoked depolarizations that result in high frequency firing \textit{in vitro}.

\textbf{3.5.2 SK currents contribute to differences in high frequency firing among the dopamine neuron subpopulations, while BK currents do not.}

In our examination of the differences in firing frequency among the dopamine neuron subpopulations, we observed a distinct AHP that followed evoked high frequency firing that is present in the mesoaccumbal and mesostriatal dopamine neurons but not the mesocortical dopamine neurons. Our work is consistent with previous work that has demonstrated an abundance of SK channels in SNc as compared to VTA dopamine neurons from immunostaining experiments, and a large amplitude evoked after-hyperpolarization current that was present following stimulation in SNc dopamine neurons as compared to VTA dopamine neurons from electrophysiology experiments (Wolfart et al., 2001). Our work shows a clear demonstration of SK mediated differences that is dopamine neuron subpopulation specific, with an abundance in the mesoaccumbal and by proxy, the mesostriatal dopamine neurons, and absence in the mesocortical dopamine neurons.

It is quite possible that in addition to the difference observed in the distribution of SK channels, there could be an accompanying difference in the distribution of the calcium channels that the calcium activated potassium channels are dependent upon. This question has been addressed among the VTA and SNc dopamine neurons but not among the dopamine neuron subpopulations. Work by Wolfart et al., has demonstrated the coupling of certain calcium ion channels to SK channels that prevent burst firing in SNc dopamine neurons – T-type calcium channels (Wolfart and Roeper, 2002). Now whether T-type calcium channels are ubiquitously expressed at similar levels among all the dopamine neurons remains unknown. Additionally, a recent report from Philippart and colleagues has demonstrated the presence of larger amplitude L-type calcium currents in SNc dopamine neurons and larger N-type calcium currents in VTA dopamine neurons (Philippart et al., 2016). As such, there exists some evidence of a differential
expression of L- and N-type calcium channels among the dopamine neurons. In the future, it will be important to clarify this question among the dopamine neuron subpopulations as it will further test the calcium hypothesis. This hypothesis posits that SNc dopamine neurons are in a vulnerable state because of the presence of larger densities of calcium channels that provide increased calcium for sustained bioenergetics and eventual mitochondrial oxidative stress which leads to their eventual death in Parkinson’s disease (Puopolo et al., 2007; Chan et al., 2009; Philippart et al., 2016; Surmeier et al., 2017).

Our work also demonstrates that BK currents do not contribute to limiting high frequency firing among the mesoaccumbal dopamine neurons. As high threshold currents, BK currents may play a major role in repolarization, particularly during pacemaking. In SNc dopamine neurons, BK currents have been demonstrated to be a larger contributor to pacemaking as opposed to high frequency firing recorded in slices (Kimm et al., 2015). It is possible that this may also be the case among the other dopamine neuron subpopulations. Our experiments only tested the contribution of BK currents to high frequency firing and not repolarization during pacemaking. In the future, it would be important to determine the differential contribution to these two modes of excitability, and to also examine whether BK channels are differentially expressed among the dopamine neuron subpopulations. As such, this remains an open question in the field. Interestingly, TRAP mRNA-seq data has yielded the differential expression of KCNMß4, a beta 4 modulatory sub-unit of the BK channel, Slo-1 (or KCNM1), at a quantity two times higher than its expression in SNc dopamine neurons (Brichta et al., 2015). This modulatory subunit, KCNMß4, has been notably found in neuronal mitochondria and one hypothesis is that it could serve in a neuroprotective capacity, guarding against excessive calcium influx (Piwonska et al., 2008). If true, this would be consistent with the resilience of VTA dopamine neurons against the events that trigger the neuronal death observed among SNc dopamine neurons in Parkinson’s disease.
3.5.3 $K_v$3 currents among the mesocortical and SNC dopamine neurons.

We reported that mesocortical dopamine neurons can fire at higher evoked firing rates and display narrow action potentials - two properties that deviate from the typical characterization of dopamine neurons (Bjorklund and Dunnett, 2007b, a). These are two excitability traits that are consistent with the reported contribution of $K_v$3 currents to the excitability properties of central neurons like those in the substantia nigra pars reticulata (SNr) and interneurons of the striatum (Lenz et al., 1994; Ding et al., 2011b). On examination of the large step depolarizations, we found that putative $K_v$3 currents were consistently present but indistinguishable among all the dopamine neuron subpopulations examined – mesocortical, mesoaccumbal and SNC. We observed the same trend with spike evoked interspike interval currents recorded in response to high frequency firing via action potential clamp. Lastly, in all cases, we observed that the proportion of TEA sensitive currents was notably less than the GTX sensitive currents. As such, under our set of conditions, we find that the putative $K_v$3 currents do not dominate the interspike interval currents during high frequency firing in mesocortical dopamine neurons.

The firing data from the mesocortical dopamine neurons presents a paradox - the spontaneous firing rate of a good number of these neurons fell within the expected range of a typical dopamine neuron – 1 to 5 Hz, but the evoked firing rates of a fraction of the neurons fall well outside the maximal 10-20 Hz range expected of most dopamine neurons. This phenomenon is unlike what is observed in most central neurons that do present with high rates of evoked high frequency firing - these neurons also display higher rates of spontaneous activity than is observed in dopamine neurons, enabled in part by the fast inactivation kinetics of the $K_v$3 current (Rudy and McBain, 2001). Our results of a low contribution of putative $K_v$3 currents to large step depolarizations and interspike interval currents during high frequency firing among the SNC dopamine neurons is consistent with past results that demonstrate a low amplitude of $K_v$3 currents that is present among the SNC dopamine neurons as compared to SNR neurons.
(Ding et al., 2011b). This has been confirmed with immunostaining data that has also demonstrated the somatodendritic presence of $K_v3.2$ and $K_v3.3$ in SNc dopamine neurons, although not comparatively to other brain regions (Dufour et al., 2014). Interestingly, previous TRAP data of mRNAs in VTA and SNc dopamine neurons, do demonstrate a larger fold change in the mRNA for $K_v3$ family members notably $KCNC1$, $KCNC2$ and $KCNC3$ ($K_v3.1$ - $K_v3.3$) among the VTA dopamine neurons as compared to the SNc dopamine neurons (Brichta et al., 2015). Despite these findings in the literature, our electrophysiological data does not reflect this differential expression. One possibility for this discrepancy could be that a different subpopulation of VTA dopamine neurons could instead express higher proportions of the $K_v3$ family members that results in their higher firing rates. Amygdala and nucleus accumbens shell- and core-projecting dopamine neurons have also been demonstrated to display fast spiking activity, although the ionic mechanisms contributing to that spiking remains to be elucidated (Lammel et al., 2008). Another possibility is that mRNA levels do not necessarily translate to protein expression level (Maier et al., 2009). As such, post translational modifications could result in the low expression of these channels despite their high mRNA levels. Lastly and more importantly, our experiments focused on the spike evoked interspike interval currents during action potential clamp. One possibility based on our experimental set-up is that the fast inactivation of $K_v3$ currents would leave little currents flowing during the interspike interval. As such, in the future, it would be paramount to carry out action potential clamp experiments in dissociated neurons that analyze the contribution of $K_v3$ currents to both single action potential and high frequency firing waveforms with a focus on the currents evoked during the action potential. This is particularly important as $K_v3$ currents are among the least studied in dopamine neurons.
3.5.4 $K_V^2$ currents among the mesocortical and SNc dopamine neurons.

$K_V^2$ currents, another of the delayed rectifier potassium currents, are also present among the SNc dopamine neurons (Dufour et al., 2014; Kimm et al., 2015). Their relatively slow activation and deactivation rates make them a plausible candidate for limiting the rate of high frequency firing among dopamine neurons (Guan et al., 2007; Liu and Bean, 2014). As such, we examined for the presence of these currents, and found that in response to both large step depolarizations and interspike interval currents elicited in response to high frequency firing, $K_V^2$ currents made up between ~40-50% of the elicited control current. This was seen in all dopamine neuron subpopulations examined. Notably, this current was also significantly larger than the amplitude of the $K_V^3$ currents except in the mesocortical dopamine neurons during large step depolarizations. A closer examination of the deactivation kinetics of the $K_V^2$ currents also revealed no difference among the dopamine neuron subpopulations. However, we did notice a difference in the build-up current (BUC) of the spike evoked interspike interval currents, with a faster build-up kinetics among the SNc dopamine neurons as compared to the unlabeled VTA dopamine neurons and mesocortical dopamine neurons. One hypothesis we set out to test was that the speed of the build-up current was related to the deactivation kinetics of the $K_V^2$ current. We found this relationship was weakly correlated, and thus likely not the chief underlying mechanism.

The phenomenon we observed of the accumulation of the spike-evoked $K_V^2$ current during the interspike interval among the mesocortical and SNc dopamine neurons is one that is strangely inconsistent with previous descriptions of the biophysical properties of the $K_V^2$ currents. Previous work on $K_V^2$ in mammalian neurons has described cumulative inactivation, slow tail deactivation, slow activation and their role in facilitating exocytosis, however we could find no mention of a cumulative activation of the $K_V^2$ current (Klemic et al., 1998; Guan et al., 2007; Feinshreiber et al., 2010). Interestingly, one study has demonstrated the presence of use-
dependent facilitation of a Kv2-like (called KVS-1) current in C. elegans which they rightfully so termed ‘cumulative activation’ (Rojas et al., 2008). The cumulative activation of the KVS-1 is voltage and time dependent. More importantly, it appears to be cell-type specific with possible modulatory subunits aiding in this peculiar property. One idea we propose to be tested in future experiments is that differential modulatory subunits possibly regulate the Kv2 channels and perhaps result in this possible ‘cumulative activation’ property that is different among the dopamine neurons. One piece of data that adds credence to this idea is TRAP-seq data that shows the presence of two of the electrically silent potassium channel subunits – KCNS2 and KCNS3 (Brichta et al., 2015). These silent modulators of Kv2 channels can form heterotetramers and shift their biophysical properties, expanding the diversity of functional potassium channels that can be expressed in an organism (Bocksteins, 2016). Accordingly, KCNS2 (Kv9.2) has been stated to be overwhelmingly expressed in the VTA, while KCNS3 (Kv9.3) is overwhelmingly expressed in the SNc (Brichta et al., 2015). As such, we propose that future experiments not only test for the presence of these two modulatory subunits among the dopamine neuron subpopulations, but also for their roles in displaying the ‘cumulative activation’ feature of the Kv2 currents observed during high frequency firing among the dopamine neurons. In preliminary experiments, we have used RNA-scope (RNA in situ hybridization) to test for the presence of KCNS3 and have found indications of it being present but sparsely distributed among the dopamine neurons (data not shown). As it stands, we currently have a knockout mouse for the KCNS3 which will be used as a tool to address the above-mentioned questions. Lastly, we also acknowledge that the extent of the assembly of these two silent channels with the Kv2 channel alpha subunits also remains an open question.

* (KVS-1 is the name of the potassium channel in the C. elegans model organism – not to be confused with the KvS electrically silent potassium channels in mammalian ion channel nomenclature)
Figure 3-1 Midbrain dopamine neuron subpopulations exhibit different firing frequencies.


D, E, F, Examples of firing evoked from a holding potential of ~ -70 mV in a D) Mesocortical E) Mesoaccumbal and F) Mesostriatal dopamine neuron.

G, Summary of average spontaneous firing rates in the mesocortical (MC) (red), mesoaccumbal (blue) and mesostriatal (black) dopamine neurons.

H, Frequency-Intensity plot of evoked firing frequencies from ~ 70 mV against the amount of injected current.

I, Plot of the maximal evoked firing frequency in each mesocortical (MC) (red), mesoaccumbal (blue) and mesostriatal (black) dopamine neuron against the amount of injected current. Maximal firing frequency is designated as the frequency the neuron is firing at right before it enters depolarization block.
Figure 3-2 Comparison of Spike Features among the Mesocortical, Mesoaccumbal and Mesostriatal dopamine neurons.


D, E, F, Examples of phase plane plots in D) Mesocortical E) Mesoaccumbal and F) Mesostriatal dopamine neuron. The y-values of the peak and trough on this plot correspond to the values of the upstroke and downstroke respectively.

G, Summary of average half-width values in a group of mesocortical (MC) (red), mesoaccumbal (blue) and mesostriatal (black) dopamine neurons. The average half-width is measured as the width of the action potential at the half-way point between the peak and trough of the action potential.

H, Summary of the average downstroke values from a group of mesocortical (MC) (red), mesoaccumbal (blue) and mesostriatal (black) dopamine neurons. Downstroke which is the rate of repolarization, is obtained from the derivative of the action potential and is where the fastest minimal change occurs. From the phase plane plot, it is the minimal y-value.

I, Plot of the average half-width against the downstroke values from a group of measured action potentials from each mesocortical (MC) (red), mesoaccumbal (blue) and mesostriatal (black) dopamine neuron. Pearson’s coefficient r-values are: 0.86 (MC, red), 0.83 (MA, blue) and 0.76 (MS, black).

J, Summary of the average trough values from a group of mesocortical (MC) (red), mesoaccumbal (blue) and mesostriatal (black) dopamine neurons.

K, Summary of the average upstroke values from a group of mesocortical (MC) (red), mesoaccumbal (blue) and mesostriatal (black) dopamine neurons. Upstroke which is the rate of depolarization, is obtained from the derivative of the action potential and is where the fastest maximal change occurs. From the phase plane plot, it is the maximal y-value.

L, Summary of the average peak values from a group of mesocortical (MC) (red), mesoaccumbal (blue) and mesostriatal (black) dopamine neurons.
Figure 3-3  Lack of effect of the D2 antagonist, Sulpiride, and GIRK blocker, Ba$^{2+}$ on mesocortical and mesoaccumbal dopamine neuron high frequency firing.

A, B, Examples of evoked maximal firing in control (no drugs in bath) A, Mesocortical B, Mesoaccumbal dopamine neurons.

C, D, Examples of evoked maximal firing evoked from a holding potential of ~ -70 mV with Barium (100 nM) and Sulpiride (10 µM) in the bath in the same C) Mesocortical and D) Mesoaccumbal dopamine neurons in A, and B, above.

E, F, Evoked maximal firing frequency in each E, mesocortical (MC) (red circles) and F, mesoaccumbal (blue circles) dopamine neuron. Values in the control condition are to the left and values in the Barium and Sulpiride conditions are on the right. Average values are indicated with error bars.

G, H, Frequency-Intensity plot of evoked firing frequencies from ~-70 mV against the amount of injected current in G, mesocortical (MC) (red circles) and H, mesoaccumbal (blue circles) dopamine neurons in both control and drug (Barium + Sulpiride) conditions. Note the overlap in the F-I curves for both the mesocortical and mesoaccumbal dopamine neurons in control and drug conditions.
Figure 3-4 Midbrain dopamine neuron subpopulations exhibit heterogeneity in an evoked after-hyperpolarization potential (eAHP).

A, B, C, Examples of evoked firing from ~70 mV depicting the eAHP that followed the high frequency firing in a A) Mesocortical B) Mesoaccumbal and C) Mesostriatal dopamine neuron. The eAHP is measured as the difference between the baseline and the minimal value of the eAHP within each neuron as shown on the examples.

D, Summary plot of the amplitude of the eAHP against the firing frequencies in a group of Mesocortical (red), Mesoaccumbal (blue) and Mesostriatal (black) dopamine neurons. The values of the AHP were first averaged in 5 Hz bins within each neuron, and then binned in 10 Hz bins across all the neurons within a subpopulation.
Figure 3-5 SK currents underlie the eAHP, and limit high frequency firing in Mesoaccumbal Dopamine Neurons.

**A, B, C,** Examples of evoked firing from ~70 mV depicting the eAHP that followed the high frequency firing in a **A)** Control Mesoaccumbal dopamine neuron **B,** Mesoaccumbal dopamine neuron with Paxilline (10 µM) and Apamin (300 nM) in the bath, **C,** Mesoaccumbal dopamine neuron with only Apamin in the bath. The eAHP is measured as the difference between the baseline and the minimal value of the eAHP within each neuron as shown on the examples.

**D,** Summary plot of the amplitude of the eAHP against the firing frequencies in a group of control Mesoaccumbal (**blue**), Mesoaccumbal in Paxilline + Apamin (**green**), and Mesoaccumbal in Apamin (**pink**) dopamine neurons. The values of the AHP were first averaged in 5 Hz bins within each neuron, and then binned in 10 Hz bins across all the neurons within a subpopulation.

**E,** Frequency-Intensity plot of firing evoked from ~70 mV in a group of control Mesoaccumbal (**blue**), Mesoaccumbal in Paxilline + Apamin (**green**), and Mesoaccumbal in Apamin (**pink**) dopamine neurons.

**F,** Example of evoked firing from ~70 mV depicting the eAHP that followed the high frequency firing in a mesoaccumbal dopamine neuron in the presence of paxilline (10 µM).

**G,** Frequency-Intensity plot of firing evoked from ~70 mV in a group of control Mesoaccumbal (**blue**), and Mesoaccumbal in Paxilline (**orange**) dopamine neurons.
Figure 3-6 Comparing Kv2 and Kv3 currents in response to step depolarizations in mesocortical, mesoaccumbal and SNc dopamine neurons.

A, B, C, Examples of step depolarizations in an example A, Mesocortical B, Mesoaccumbal and C, SNc dopamine neuron, with black representing control, dark green representing Guangxitoxin (GTX) (200 nM) and light green representing the subtraction of the control and GTX traces also called GTX sensitive.

D, E, F, Examples of step depolarizations in an example A, Mesocortical B, Mesoaccumbal and C, SNc dopamine neuron, with black representing control, dark brown representing Tetraethylammonium (TEA) (1 mM) and dark yellow representing the subtraction of the control and TEA traces also called TEA sensitive.

G, H, I, Summary showing the ratio of currents that are blocked, numbers were averaged and then normalized to the average control current amplitude. G, mesocortical (MC), H, mesoaccumbal (MA) and I, SNc dopamine neurons. Asterisk means p-value < 0.05.
Figure 3-7 Comparing spike evoked interspike interval $K_{V2}$ and $K_{V3}$ currents in mesocortical and SNc dopamine neurons in response to a waveform previously recorded in a Mesocortical dopamine neuron.

A, B, Examples of currents evoked in response to high frequency firing from a previously recorded mesocortical and SNc dopamine neuron (top) in a A, Mesocortical and B, Mesostriatal dopamine neuron. Same as the top but with a higher magnification on the currents. Black, control condition; green, in GTX and dark yellow, in TEA.

C, D, Summary plot of the average of the last two ISIs currents in response to high frequency firing. Black circles, control condition; green circles, in GTX and dark yellow circles, in TEA. Opaque circles represent the average values with error bars. C, Mesocortical and D, Mesostriatal dopamine neurons.

E,F, Summary showing the ratio of currents that are blocked, numbers were averaged and then normalized to the average control current amplitude. E, mesocortical (MC), F, SNc dopamine neurons.
Figure 3.8 Comparing spike evoked interspike interval $K_{V2}$ and $K_{V3}$ currents in mesocortical and SNc dopamine neurons in response to a waveform previously recorded in a SNc dopamine neuron.

A, B, Examples of currents evoked in response to high frequency firing from a previously recorded mesocortical and SNc dopamine neuron (top) in a A, Mesocortical and B, Mesostriatal dopamine neuron. Same as the top but with a higher magnification on the currents. Black, control condition; green, in GTX and dark yellow, in TEA.

C, D, Summary plot of the average of the last two ISIs currents in response to high frequency firing. Black circles, control condition; green circles, in GTX and dark yellow circles, in TEA. Opaque circles represent the average values with error bars. C, Mesocortical and D, Mesostriatal dopamine neurons.

E, F, Summary showing the ratio of currents that are blocked, numbers were averaged and then normalized to the average control current amplitude. E, mesocortical (MC), F, SNc dopamine neurons.
Figure 3-9 Build-up of the spike evoked interspike interval $K_v^2$ currents in mesocortical, mesoaccumbal and SNc dopamine neurons.

A, B, C, Examples of GTX sensitive, $K_v^2$ currents in response to a previously recorded high frequency firing train in a mesocortical dopamine neuron that is used as the command voltage in a A, mesocortical, B, VTA and C, SNc dopamine neuron. Dotted lines are single exponential fits to the build-up current (BUC) during the interspike interval.

D, E, F, Step depolarizations from 0 mV to -50 mv (100 ms) to allow for measurement of the deactivation kinetics of the $K_v^2$ current in a D, mesocortical, E, VTA and F, SNc dopamine neuron. Dotted lines are exponential fits to the decay of the current.

J, Summary depicting the values of time constant of the build-up current in each mesocortical (red), VTA (purple) and SNc (black) dopamine neuron. Averages with the error bars are shown in open circles.

K, Summary depicting the values of time constant of the tail current (deactivation kinetics) in each mesocortical (red), VTA (purple) and SNc (black) dopamine neuron. Averages with the error bars are shown in open circles.

L, Plot of the relationship between the time constant of the build-up current and the time constant of the tail current from the step depolarization. There is a weak to moderate negative relationship between the two variables, with $P_r = -0.366$. 
CHAPTER 4

CONCLUSIONS AND FUTURE DIRECTIONS
4.1 SUMMARY

Initial studies on midbrain dopamine neurons depicted them as functionally homogeneous, with the ubiquitous role of mediating reward signaling. However, we now know that midbrain dopamine neurons mediate a wide range of behaviors essential to an organism’s survival that go beyond reward signaling, and include motivation, aversion and movement initiation amongst others. Dopamine neurons are also involved in a wide range of disorders including Parkinson’s disease, addiction, schizophrenia and attention deficit hyperactivity disorder (ADHD) to mention a few. The involvement of dopamine neurons in a vast array of behaviors and disorders underscores the vital role of these neurons in the brain. The discovery the dopamine neurons participate in different brain circuits, and therefore can support several different behaviors based on their axonal projections to disparate brain regions allowed for the classification of the dopamine neurons into subpopulations. These dopamine neurons subpopulations are functionally heterogeneous, and yet, a clear demonstration of the ionic mechanisms that underlie the heterogeneity in their functional intrinsic properties is still lacking.

The firing pattern of midbrain dopamine neurons determines the information that is relayed to downstream neurons in the circuit. Both tonic firing, that determines ambient dopamine, and phasic firing, that determines the high amplitude synaptic release of dopamine, are essential features of dopamine neuron function. Additionally, their quiescent period or pause is an important conveyer of reward omission and aversion in some cases. Indeed, several studies have elucidated the behavioral correlates of the different modes of dopamine neuron excitability. Furthermore, the ionic mechanisms that underlie the different excitability states have been studied to an extent. However, most of these studies have been carried out in the context of the two broad groups of dopamine neurons anatomically located into the SNc and the VTA. Given the growing knowledge on the specificity of the dopamine neuron subpopulations in the behaviors they mediate, the pathologies they underlie and the differences in their excitability, it is essential to ask these questions in the context of the dopamine neuron subpopulations.
Indeed, uncovering the biological principles that underlie the diversity of dopamine neuron subpopulations will provide insight into molecules that can be targeted in disease models of dopamine neuron subpopulations. In my dissertation, I focus on pauses, pacemaking and high frequency firing – all excitability patterns of dopamine neuron subpopulations that have been observed in vivo in the literature and can be observed and elicited in vitro. First, I examined and compared pauses in mesoaccumbal and nigrostriatal dopamine subpopulations. Next, I examined high frequency firing in mesocortical, mesoaccumbal and mesostriatal dopamine subpopulations. In both projects, I examined the ionic mechanisms that contribute to the differences I observed in those excitability states.

I examined and compared pauses evoked by current injections in both mesoaccumbal and nigrostriatal dopamine subpopulations in chapter 2. We also combined synaptic stimulations, GABA uncaging and computational modeling to further examine pauses and the contributing ionic mechanisms. We provide ample evidence of pauses that are temporally different, even at physiological voltages. While mesoaccumbal dopamine neurons displayed long duration pauses, nigrostriatal dopamine neurons displayed faster pauses. The difference in the pauses or rebound delays were temporally dependent on the duration of hyperpolarization. Furthermore, these differences could be evoked at physiological voltages. More importantly, we found these pauses to be sensitive to the specific $I_A$ blocker AmmTX3, even when evoked synaptically or by GABA uncaging. Underlying the difference in the pauses were longer inactivation kinetics of the $I_A$, combined with small amplitude $I_H$. We used a computational model of the dopamine neurons to demonstrate that even increasing the amplitude of $I_H$ and T-type calcium currents, both subthreshold depolarizing currents that oppose the action of $I_A$, in a model of a mesoaccumbal-like dopamine neuron, could not significantly override the quiescence induced by the slow $I_A$ inactivating kinetics. As such, the combination of slow inactivating $I_A$ and low amplitude $I_H$ currents in the mesoaccumbal dopamine neurons predispose them to be more sensitive to hyperpolarizing stimuli.
In chapter 3, I revisit the phenomena of differential high frequency firing among the dopamine neuron subpopulations, with the goal of uncovering the high threshold potassium currents that enable such differences. I recapitulate previous findings that demonstrate that mesocortical dopamine neurons fire at higher maximal firing frequencies and I carried out a more thorough and comparative analysis of the spike properties. Indeed, mesocortical dopamine neurons do fire at higher maximal frequencies, but unlike previous literature, I find that their average pacemaking frequencies still falls within the expected range of the “typical” dopamine neuron (i.e. comparable to mesostriatal). More importantly, I found narrower action potential spikes and faster rates of repolarization among the mesocortical dopamine neurons as compared to mesoaccumbal and nigrostriatal dopamine neurons. I tested the widely-held notion in the literature that the ambient dopamine levels from somatodendritic release limits the maximal firing rate of dopamine neuron that possess D2 receptors. I found this notion to not be the case under my test conditions. I examined the contribution of calcium-activated potassium currents, and found that SK currents limited high frequency firing among the mesoaccumbal dopamine neurons, in addition to enhancing the amplitude of the AHP that followed high frequency firing, this AHP was absent in mesocortical dopamine neurons. As such, the differential expression of SK channels is one contributing factor that enables the heterogeneity in firing among the dopamine neuron subpopulations. Using action potential clamp, I examined the interspike interval currents flowing during high frequency firing that were sensitive to GTX and 1mM TEA - $K_v2$ and $K_v3$ currents respectively. I found that the comparative proportions of the $K_v2$ currents was not different among the mesocortical, mesoaccumbal and SNc dopamine neurons. The same was the case for the $K_v3$ currents. Interestingly, we did find evidence of a differential build-up of the interspike interval currents that is $K_v2$ mediated. We also provide evidence that this $K_v2$ build-up current could not be explained by the deactivation kinetics of the $K_v2$ current.
4.2 FURTHER ELUCIDATING THE UNDERPINNINGS OF PAUSES IN MIDBRAIN DOPAMINE NEURON SUBPOPULATIONS.

Evidence from in vivo experiments has demonstrated that dopamine neurons pause in response to either reward omission and/or the presentation of aversive stimuli. As such, the pause synaptically translates into a dramatic reduction in the release of dopamine. Our work presents a differential characteristic of the pause in mesoaccumbal and nigrostriatal dopamine neurons, with longer pauses among the mesoaccumbal as compared to the nigrostriatal dopamine neurons. We find that underlying this feature is a combination of differences in the inactivation kinetics of $I_A$ and the amplitude of $I_H$. This highlights an additional layer of diversity among the dopamine neuron subpopulations, in which a difference abounds in the biophysical property of the ionic channels that underlie the pauses, as opposed to a simple reduction in the quantity of the ion channels (as seen with $I_H$). As such, our work reinforces the idea of heterogeneity among the midbrain dopamine neuron subpopulations that exists even at the cellular level. This argues for more work that takes the diversity of the dopamine neurons into consideration when questions on their functional roles are asked. However, while we do present evidence of the ionic mechanisms that contribute to such differences, there are still several questions that remain unanswered.

Our work demonstrated the role of the inactivation kinetics of $I_A$ in determining the duration of pauses among mesoaccumbal and nigrostriatal dopamine neurons. However, the molecular underpinning that contributes to the differences in the inactivation kinetics of $I_A$ among the dopamine neuron subpopulations is an outstanding question that remains unanswered. Previous work has demonstrated the contribution of two auxiliary proteins, $K_V$ channel interacting proteins (KChIPs) and dipeptidyl peptidase-like proteins (DPPL), to modifying the biophysical properties of $K_V$4 channels. KChIPs are cytoplasmic calcium binding proteins that significantly increase the surface expression of $K_V$4 channels (An et al., 2000; Jerng et al., 2004b). There are four members of the KChIP family – KChIP1-KChIP4, with different isoforms
and varying expression patterns in the brain (Jerng and Pfaffinger, 2014). They modify the gating kinetics of Kv4s by sequestering their N-terminus in the cytoplasm; and increase channel surface expression by masking the endoplasmic retention signal on Kv4 proteins (Jerng et al., 2004b; Tang et al., 2013). In general, KChIPs 1-3 have been found to increase Kv4 amplitude, slightly reduce the inactivation kinetics and increase the rate of recovery from inactivation, while KChIP4 has a range of different effects depending on its spliced isoform (Holmqvist et al., 2002; Baranauskas, 2004; Wang et al., 2007; Wang, 2008). For example, a combination of RT-PCR and patch-clamp electrophysiology data has shown that the KChIP4A isoform co-localizes with Kv4 channels and underlies the slowly inactivating A-type potassium currents observed in globus pallidus neurons, similar to what we observed in our experiments in mesoaccumbal dopamine neurons (Baranauskas, 2004). Past evidence shows that KChIP4 binds to the N-terminus of Kv4 channels, thereby affecting their movement and can occlude the ion pore (Gebauer et al., 2004). As such, one proposed suggestion is that the expression of the KChIP4A isoform could be a major determinant in the differences observed in the inactivation kinetics of IA among the dopamine neuron subpopulations. However, there is a possibility that the mechanisms underlying the inactivation kinetics of IA is more nuanced than that data suggests. Data from single-molecule subunit counting in mammalian cells transfected with different stoichiometric ratios of Kv4 and KChIP4 demonstrated that different KChIPs can compete for the KChIP binding sites on the Kv4 channel (Zhou et al., 2015). Transfections of KChIP4A and Kv4 in a 4:4 manner showed a dramatic slowing in the speed of the Kv4.3 current, compared to the combination of KChIP4A and KChIP4bL isoforms with the Kv4.3 channel (Kitazawa et al., 2014; Zhou et al., 2015). As such, the stoichiometric assembly of the KChIPs and Kv4 channels can expand the range of biophysical properties that Kv4 mediated IA currents can display. Recent TRAP mRNA data from the dopamine neurons demonstrate an increased expression of KChIP1 and 2 mRNA among VTA dopamine neurons, but an increased expression of KChIP3 among SNc dopamine neurons. As such, an alternative suggestion to the
KChIP4A hypothesis is that could be the differential stoichiometric combination of KChIP1-4 among the dopamine neurons, with the possibility that KChIP1 and 2 occupy more binding sites on Kv4 channels in the VTA, and KChIP3 occupy more binding sites on Kv4 channels in the SNc. This way, a gradient of different biophysical properties of the IA is established across the dopamine neurons, with some properties being more dominant among certain dopamine neuron subpopulations as we observed in the mesoaccumbal and nigrostriatal dopamine neurons in our experiments. Further experiments are needed to elucidate the biophysical modalities at play in the IA channel differences between mesoaccumbal and nigrostriatal dopamine subpopulations.

DPPLs are another auxiliary subunit that modify Kv4 channel physiology. These are integral membrane proteins belonging to the dipeptidyl aminopeptidase family, with wide expression in the brain and other areas of the body (Jerng et al., 2004a). DPPLs bias the voltage dependence of activation and inactivation of Kv4.2 and Kv4.3 towards hyperpolarized values (Jerng et al., 2007; Nadal et al., 2006). It remains unclear how the DPPL proteins contribute to the differences observed in the biophysical properties of IA in the midbrain dopamine neuron subpopulations.

Lastly, we show that IA can facilitate the duration of quiescence in synaptically induced and GABA uncaged pauses in the dopamine neurons. We demonstrated that the synaptically evoked pauses were mediated by both GABA_A and GABA_B receptors. However, it would be interesting to know if the enhancement of inhibition by IA in the mesoaccumbal and nigrostriatal dopamine neurons occurs selectively via the activation of either GABA_A or GABA_B receptors in these two subpopulations. Recent work has demonstrated the selective innervation of accumbal inputs onto VTA dopamine neurons via GABA_B receptors (Edwards et al., 2017). Further work should examine if this is true among all the VTA dopamine neurons receiving inhibitory inputs or if it is specific to certain dopamine subpopulations.

Past work has demonstrated that the reversal potential of Cl⁻ is depolarized (~ -60 mV) in the dopamine neurons (Gulacsi et al., 2003). Indeed, we demonstrate that differential pauses
can be elicited within the physiological voltage range, as depolarized as -55 mV. As such, in general, hyperpolarization by GABA\textsubscript{A} currents could be more difficult to obtain as compared to GABA\textsubscript{B} currents. This phenomenon might argue for more GABA\textsubscript{B} synapses onto the dopamine neurons as compared to GABA\textsubscript{A}. However, the whether this is true and/or different among the dopamine neuron subpopulations remains to be tested.

### 4.3 Further Uncovering the Elements of Differential Firing Among the Midbrain Dopamine Neuron Subpopulations, and Technical Considerations.

Mesocortical dopamine neurons fire at higher tonic and phasic rates as previously demonstrated in \textit{in vivo} and \textit{in vitro} studies. I demonstrate that a higher rate of pacemaking and high frequency firing is present among the mesocortical dopamine neurons, and extend the work of others by uncovering the ionic mechanisms that enable such high frequency firing. Our work demonstrates that differences in ambient and endogenous D2R currents do not underlie the differences in high frequency firing. However, we do find differences in SK currents which limits high frequency firing among the mesoaccumbal dopamine neurons. Furthermore, for the first time, we find a temporal difference in the build-up of K\textsubscript{V}2 mediated interspike interval currents, that is slower in mesocortical and unlabeled VTA dopamine neurons as compared to SNc dopamine neurons. Our work reconfirms previous findings in the literature and highlights a possible new role of a differential property of K\textsubscript{V}2 currents among the dopamine neuron subpopulations. However, further work is needed to elucidate this mechanism.

Experiments were carried out to dissect the high threshold potassium currents that contribute to high frequency firing among the mesocortical, mesoaccumbal and mesostriatal dopamine neurons. These voltage clamp experiments were performed in neurons in intact tissue. The undetached processes predispose the recorded ionic currents to the factor of space clamp, and is a technical limitation for these set of experiments. One argument that slightly alleviates this concern is evidence that shows mostly proximal somatodendritic distribution of
Kv2 currents in the dopamine neurons, as such, our currents in these regions could be expected to be reasonably clamped (Dufour et al., 2014). Secondly, previous work from our group and others has demonstrated the tight electronic coupling between the soma and dendrites in dopamine neurons such that somatic depolarizations can enhance synaptically evoked calcium signals at dendritic distances considered distal (Hausser et al., 1995; Gentet and Williams, 2007; Hage and Khaliq, 2015). Despite this, future experiments will should be carried out in dopamine neuron subpopulations that have been enzymatically dissociated from their large processes. However, one limitation of this is that the dissociation process will result in a further reduction in the number of retrogradely labeled dopamine neurons, which are a fewer number to begin with. Alternatively, current recordings can be obtained from nucleated patches from dopamine neuron subpopulations. Overall, it is essential that future experiments recapitulate the findings in the dopamine neuron subpopulations obtained above under more ideal voltage clamp settings.

While we demonstrated a difference in the Kv2 mediated interspike interval currents, further experiments are needed to elucidate the source of this difference among the VTA and SNc dopamine neurons. Our experiments demonstrate a difference in the build-up and the non-dependence of this phenomenon on the deactivation kinetics of Kv2. In the future, it will be important to examine if this contribution stems from silent auxiliary subunits as previously proposed (see chapter 3 discussion). Indeed, this discovery will propose a novel and additional insight into the diversity of the ion channels that mediate differential firing among the dopamine neuron subpopulations.

In conclusion, this dissertation adds further insight into the underlying mechanisms that support the diversity in the excitability of midbrain dopamine neuron subpopulations. In the first study, we demonstrate the presence of different durations of rebound pauses, even at physiological voltages, among the mesoaccumbal and nigrostriatal dopamine neurons and shed light on how it is a combination of differential IA inactivation kinetics and IH amplitude that
supports this difference. In the next study, we show that mesocortical, mesoaccumbal and mesostriatal dopamine neurons do display differential pacemaking and high frequency firing rates, wherein differences in SK currents and the build-up of a $K_v2$ interspike interval current could be responsible for these differences. As more light is shed on the heterogeneity of the dopamine neurons at the cellular level, more protein targets that are selectively present in different subpopulations will be identified. These targets could aid in the isolation of the dopamine neuron subpopulations for treatments specific to the diseases that selectively affect these subpopulations. Furthermore, more studies like this will ultimately lead to a better understanding of how the different dopamine neuron subpopulations integrate information as they participate in specific and disparate circuits.
Bibliography:


Lacey MG, Mercuri NB, North RA (1987) Dopamine acts on D2 receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. J Physiol 392:397-416.


Paladini CA, Tepper JM (1999) GABA(A) and GABA(B) antagonists differentially affect the firing pattern of substantia nigra dopaminergic neurons in vivo. Synapse 32:165-176.


Tong ZY, Overton PG, Clark D (1996a) Antagonism of NMDA receptors but not AMPA/kainate receptors blocks bursting in dopaminergic neurons induced by electrical stimulation of the prefrontal cortex. J Neural Transm (Vienna) 103:889-904.


