NEUROBIOLOGICAL AND BEHAVIOURAL EFFECTS OF CHRONIC ADMINISTRATION OF THE D_{2/3} AGONIST ROPINIROLE IN RELATION TO IATROGENIC GAMBLING DISORDER

by

Mélanie Tremblay

B.A. (Hon), The University of British Columbia, 2011
M.A., The University of British Columbia, 2013

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES
(Psychology)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

November 2017

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Abstract

Neurobiological changes in Parkinson’s Disease (PD) involve dramatic loss of dopamine neurons in the substantia nigra (SNC) and terminals in the dorsal striatum. L-DOPA, first line treatment for PD, can produce debilitating side-effects like dyskinesia over time. Preferential dopamine D2/3 agonists like ropinirole, are used to treat PD, but these newer drugs can lead to impulse control disorders (ICDs) and gambling disorder in a significant minority of patients. The mechanism mitigating dopamine replacement therapy (DRT)-induced ICDs, or whether premorbid behavioural tendencies are a risk factor are unknown. We show that chronic ropinirole increases preference for uncertainty on the rodent Betting task (rBT), regardless of animals’ baseline preference for the safe or uncertain option. Comparatively, ropinirole had subtle effects on choice of a cued version of the rat Gambling task (rGT), while increasing impulsivity, suggesting different neural mechanisms in performance of these tasks. GSK3β has been involved in disorders of impulsivity suggesting a potential intracellular mechanism for DRT-ICDs. However, chronic administration of SB 216763, a GSK3β inhibitor, did not attenuate gambling-like behaviours following ropinirole, but also did not reliably decrease GSK3β levels, such that we were unable to unequivocally determine its role in ropinirole-induced preference for uncertainty. The “overdose” hypothesis, in which DRTs would replenish dopamine stores in the deteriorated dorsal striatum and improve movement, but overwhelm the mostly spared mesolimbic reward system, was suggested to explain DRT-ICDs in PD. However, newer studies suggest a potential involvement for the nigrostriatal, rather than mesolimbic, pathway in gambling disorders. We therefore manipulated the nigrostriatal pathway using designer receptors exclusively activated by designer drugs (DREADDs). Activation of
dopamine neurons in the SNc increased preference for uncertainty on the rBT in wager-sensitive rats, partially mimicking the effect of ropinirole, while inhibition of these neurons had no effect on the increase in preference for uncertainty caused by ropinirole. These studies suggest a potential role for activation of the nigrostriatal pathway in DRT-ICDs. They also suggest that ICDs result from DRT alone, rather than from an interaction between medication and basal risk preference, or change in dopamine function caused by the diseases for which they are prescribed.
Lay Summary

Dopamine D\textsubscript{2/3} agonist replacement therapies (DRT), like ropinirole, are newer drugs used to treat Parkinson’s disease (PD), but can lead to impulse control disorders (ICDs) including gambling disorder. The biological basis of DRT-ICDs, and whether their onset can be predicted by risky cognitive-behavioural traits, are unknown. We attempted to parse the relative contribution of dopamine loss caused by PD, DRT, and risk tendencies in developing DRT-ICDs using rodent tasks that assess gambling-like behaviours. We also tried to understand the implication of an intracellular signaling cascade implicated in disorders of impulsivity. Finally, we investigated the putative role the nigrostriatal dopamine pathway, implicated in PD, plays in DRT-ICDs using novel chemogenetic techniques. We determined that increased dopamine release within this pathway mimics the behavioural effect of ropinirole, and that ICDs likely result from DRT alone, rather than from interactions between medication and risk preferences, or changes in dopamine function caused by PD.
Preface

This dissertation is an original intellectual product of the author, Melanie Tremblay, under the supervision of Dr. Catharine Winstanley.


The osmotic pump implantation in healthy rats in Chapter 3 section 3.2.2 were performed by the author and Jay Hosking. Pump removal was performed by the author. Bilateral dorsolateral striatal 6-OHDA lesions and osmotic pump surgeries in Chapter 3.2.3 were performed by the author and Mason Silveira.

In Chapter 4 section 4.2.3, osmotic pump surgery in animals from the standard rGT was performed by the author and Jay Hosking. Surgeries in animals from the cued rGT was performed by Michael Barrus and Paul Cocker. Training of some of the animals in the cued rGT was performed by the author and Michael Barrus.

Osmotic pump surgery in Chapter 5 section 5.2.4 was performed by the author and Paul Cocker. All injections in section 5.2.3 and 5.2.5 were performed by the author.
Immunohistochemistry for tyrosine hydroxylase on brain slices from the animals who received 6-OHDA surgery in Chapter 3 section 3.2.3 and western blot in brains sections from the GSK3β experiment in Chapter 5 section 5.2.7 were performed by the author and Sukhbir Kaur.

All AAV infusion surgery in Chapter 6 section 6.2.3 and 6.2.4 were performed by the author and Mason Silveira. Mason Silveira also helped with osmotic pump implantation in section 6.2.3. Injections in the chronic CNO experiments in section 6.2.3 and 6.2.4 were performed by the author. Syringes from these experiments were prepared by the author, and with the help of Sherry Zhao, Mason Silveira, Brett Hattaway, Brittney Russell, Sukhbir Kaur and Jacquie Ferland. The forelimb adjustment step test was performed by the author, with the help of Brittney Russell. Sukbir Kaur performed genotyping and immunohistochemistry in Chapter 6 section 6.2.2 and 6.2.5.

All other procedures, including drug preparation, animal training and injections included in this dissertation were performed by the author. The author also performed all data and statistical analysis, made all figures and tables and wrote the manuscript. Revisions and contributions were provided by Dr. Catharine Winstanley, Dr. Liisa Galea and Dr. Lawrence Ward.

All procedures were in accordance with the Canadian Council on Animal Care and the University of British Columbia Animal Care Committee (ACC) and were covered by ACC protocols number A08-0519 and A13-0011.
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<tbody>
<tr>
<td>5CSRT</td>
<td>5 Choice Serial Reaction Time Task</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
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<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
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<td>AAV</td>
<td>adeno-associated virus</td>
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<td>AC</td>
<td>adenylyl cyclase</td>
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<td>ACC</td>
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<td>Akt</td>
<td>protein kinase B</td>
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<td>ANOVA</td>
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<td>cAMP response element binding protein</td>
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<td>dopamine dysregulation syndrome</td>
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<tr>
<td>Abbreviation</td>
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<td>DMSO</td>
<td>dimethyl sulfoxide</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>DREADD</td>
<td>designer drug exclusively activated by designer drug</td>
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<td>DRT</td>
<td>dopamine replacement therapy</td>
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<td>DSM-V</td>
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<td>fMRI</td>
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<td>GABA</td>
<td>gamma-aminobutyric acid</td>
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<td>IP</td>
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<td>KI/KO</td>
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<td>L-DOPA</td>
<td>L-3,4-dihydroxyphenylalanine</td>
</tr>
<tr>
<td>MAO</td>
<td>monoamine oxidase inhibitor</td>
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<tr>
<td>ML</td>
<td>medial-lateral</td>
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<td>mPFC</td>
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<td>SEM</td>
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<td>SNc</td>
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<td>substantia nigra pars reticulata</td>
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<td>STN</td>
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<td>TH</td>
<td>tyrosine hydroxylase</td>
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<td>VTA</td>
<td>ventral tegmental area</td>
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<td>WCST</td>
<td>Wisconsin card sorting test</td>
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</table>
Acknowledgements

I would like to offer my sincere thank you to my supervisor, Dr. Catharine Winstanley who has supported me and encouraged me for the duration of my graduate studies. Catharine, you have believed in me when I doubted myself and I feel I have grown so much being a part of your lab. Your “French” expressions and passionate discussions always relieved some stress in lab meetings and made me smile. Also, your knowledge and passion for research was truly inspiring and I feel so lucky to keep learning from you.

Jacquie, thank you for being such an inspiring scientist. You have a spark for research that I believe will bring you where you want to go in this field. Also, you became a real friend to me inside and outside the lab. I am grateful for all the times you told me to stop working and welcomed me to your house with a nice glass of wine, in times when we were less encouraged, and others like when we celebrated your, then my passing of comps. I look up to you for so many things.

Mason, we spend so much time together in the lab, and I know I made you laugh so many times with my funny personality, where to start? You are like my lab “bro”. Your dedication and willingness to help is truly remarkable. I really enjoyed working with you each time we did. Your professionalism, desire to do your best, and easy to work with personality always made you a person of choice with whom I always wanted to collaborate in my experiments. Sincerely thank you for all your help. You made life in the lab so much easier and were always where you were needed without even asking, you truly made my days easier.. and that always with a smile.
Thank you. I hope you remember to spend some time outside you natural habitat, the lab, in the upcoming years.. see how funny I am? ;)

Celine, thank you so much for taking care of my remaining rats for the ongoing experiment so I could write this dissertation. Trusting you was easy. Also thank you for your help in cutting brains. I really admire your endless willingness to work and help others, it is commendable.

I also want to thank our laboratory technicians and veterinarian. Shelly, thank you for your openness and availability. You have been so helpful to me with guiding me taking good care of my bébés and answering questions throughout the years. Also, thank you for making me a lucky goose to help me break a strike of all the suspicious things happening to me. It didn’t really work, but oh well ;) It remains in the lab. Also, thank you to all our lab techs for making sure our animals are in good conditions and for helping us conduct good experiments.

I would like to also offer my gratitude to the faculty, staff and fellow students at the University of British Columbia, who inspire me by their perseverance and dedication to their specific field of study, and motivated me with your enthusiastic talk about your passion for research. I also want to thank my committee members for challenging me and pushing me to learn material outside of my specific field. I also thank all my past and present labmates who have helped me learn the various skills and techniques necessary to conduct the experiments in this dissertation. Some of you became really good friends, thank you for your support.
Finally, I want to thank all my rats, my “bēbē”s, who gave their life for these experiments in hope to advance science. Without you, this work would not have been possible. Bēbēs, even when I found the hours long in the lab, your infinite cuteness and blind trust in me always made me feel that you deserved I give you my best. RIP.
Dedication

To patients with Parkinson's Disease and other diseases for which we still do not have a cure. Particularly, this dissertation is dedicated to those who develop devastating impulse control disorders (ICDs) following initiation of the highly effective dopamine $D_2/3$ agonist therapies (DRT), and are left with little alternatives. In hope that the work included here brings some understanding of the mechanisms by which DRTs trigger ICDs, and help us develop strategies to mitigate these unwanted side effects while preserving the benefits this drug class provides in relieving the motor symptoms of PD.
Chapter 1: General introduction

1.1 Parkinson’s Disease and impulse control disorders

Parkinson’s Disease (PD) is one of the most common neurodegenerative diseases in the aging population, affecting 1-2% of individuals over the age of 65 (Alves et al, 2008). Men are also 1.5 times more likely to be affected than women (see Pavon et al, 2010 for review). Slowness of movement, a difficulty initiating those movements (bradykinesia), rigidity, tremor at rest, and gait disturbances, are the hallmark symptoms of PD, although other symptoms such as constipation and loss of olfaction are believed to occur many years before the first motor symptoms of PD are observable and the disease is diagnosed (i.e. Poewe et al, 2017). A substantial loss of dopamine (3-hydroxytyramine) neurons in the substantia nigra pars compacta as well as loss of dopamine terminals and dopamine metabolites in the striatum is believed to be at the origin of the motor symptoms of PD (Ehringer and Hornykiewicz, 1960). As the disease advances, other symptoms may also occur such as cognitive dysfunction, depression, anxiety, and sleep disturbances, although other neurotransmitter systems appear to be responsible for these symptoms. For example, norepinephrine neuronal death can also be observed in humans with advanced PD (Chaudhuri et al, 2006; Halliday et al, 1990; Zarow et al, 2003). Also, loss of serotonergic signaling has been observed in PD patients and aberrant dopamine release from serotonin neurons has been involved in the development of dyskinesia in relation to the use of levodopa (L-3,4-dihydroxyphenylalanine; L-DOPA) in both humans and animals (e.g. Carta et al, 2007; de la Fuente-Fernandez et al, 2004; Fox et al, 2009; Gantz et al, 2015; Politis et al, 2014; Roussakis et al, 2016).

The neuropathology of a common form of familial PD includes the accumulation of intra-
neuronal protein aggregates such as Lewy bodies. It has been suggested that PD may be a prion
disease, in which misfolded proteins such as α-synuclein would spread to cells in the peripheral
and the central nervous system and ultimately lead to neuronal cell death. These misfolded
protein aggregates have been observed in dopaminergic cells of the substantia nigra and other
cells of the central and peripheral system (see Olanow and Brundin, 2013 for review). From this
perspective, synucleinopathy would spread from the periphery to the brainstem and cortex,
leading to the loss in function observed in PD and explains symptoms such as constipation and
loss of olfaction occurring years before PD is diagnosed, as well as the progression of common
symptoms of the disease (Braak et al, 2003). However, propagation of misfolded proteins does
not always correlate to the loss of function associated with what would be expected from the
progression of these proteins, suggesting that synucleopathy may not solely explain the
symptomatology of PD (Burke et al, 2008).

L-DOPA, a precursor for dopamine synthesis and the first line treatment for PD, has a
limited efficacy window, and the likelihood of developing debilitating side-effects such as
dyskinesia increases dramatically over time. Also, a proportion of patients on this drug develop
a syndrome known as dopamine dysregulation syndrome (DDS) defined as a compulsive use of
dopamine replacement therapy (DRT) at a dose well above what is required to control the motor
symptoms, similar to behaviours observed in drug addiction (Cantello, 2000; Giovannoni et al,
2000). More recently, preferential dopamine D2/3 family receptor agonists such as the indolinone
derivative ropinirole and the tetrahydrobenzothiazole pramipexole have been developed and used
as monotherapy or adjunct treatments for PD. Although highly effective at ameliorating the
motor symptoms of Parkinson’s disease (PD), these DRTs can engender devastating psychiatric
complications such as gambling disorders (GD) and other impulse control disorders (ICDs) in a
significant minority of patients (16-20%). Such ICDs may appear de novo and result in significant psychosocial distress and serious impairments in quality of life that can be as devastating as, or even surpass the disabilities due to, the original Parkinsonism these drugs were designed to treat (Voon et al, 2011b; Weintraub et al, 2010; Weintraub and Potenza, 2006a; Weintraub et al, 2006b). It is unclear if the change in risky choice in PD patients may be attributed to a combination of premorbid factors and DRT, or to individual differences in the physiological reaction to DRT that may depend on, or be independent from, PD.

Nonetheless, DRTs can precipitate ICDs in other patient groups for which they are prescribed, including restless leg syndrome and fibromyalgia (Holman, 2009; Quickfall and Suchowersky, 2007; Tippmann-Peikert et al, 2007; Voon et al, 2011c). Although the incidence is less frequent, likely due to the lower doses of DRT used, such findings indicate that DRT-induced ICDs (DRT-ICDs) may not be attributable to the altered brain neurochemistry caused by PD, but instead reflect a side-effect of DRTs themselves. Discontinuing DRT use typically leads to resolution of the ICD, but can leave the patient without adequate relief from their primary motor impairments. Dopamine agonist withdrawal syndrome (DAWS), a severe, stereotyped cluster of symptoms including panic attacks, dysphoria, drug craving and suicidality, has also been reported following withdrawal of DRTs (Edwards, 2013; Nirenberg, 2013; Rabinak and Nirenberg, 2010). Dopamine agonist withdrawal does not respond to alternative dopaminergic medication, and there are currently no known treatments beyond restoration of DRT. Although effective in the treatment of movement disorders, some neurologists may therefore hesitate to use DRTs for fear of initiating such a catastrophic cascade of side-effects to which a minority of patients appear vulnerable. It would obviously be of enormous value if those at risk of DRT-ICDs could be reliably identified before treatment was initiated.
It has been suggested that a prior or family history of ICDs and addiction disorders may play an important role in conferring vulnerability (Voon et al, 2007), although this does not account for every case (Voon et al, 2011d). Other studies report that DRT-induced ICDs can develop even in Parkinsonian individuals with an introverted and conservative personality, a personality that may even result as a consequence of the disease itself (Dagher and Robbins, 2009). The mechanism by which DRT can trigger GD is still unknown, yet such information could prove vital in developing strategies to mitigate such unwanted side effects while still preserving the benefits this drug class provides in relieving the motor symptoms of PD.

1.2 Dopamine and the basal ganglia

Dopamine, the major neurotransmitter involved in PD, is involved in a variety of brain functions such as reward signaling, attention, working memory, and voluntary movements, the latter being particularly associated with PD (Korchounov et al, 2010). Dopamine is created when tyrosine is metabolized by the enzyme tyrosine hydroxylase and turned into L-DOPA, which is then metabolized by the enzyme DOPA decarboxylase and turned into dopamine. Dopamine neurotransmission is modulated mostly by GABAergic (gamma-aminobutyric acid) and glutamatergic neurons, but also by cholinergic neurons. In the basal ganglia, dopamine is released from two nuclei; the ventral tegmental area (VTA) and the substantia nigra (SN). From these nuclei, dopamine reaches various areas of the basal ganglia and the limbic system such as the thalamus, nucleus accumbens, amygdala, striatum (caudate and putamen), and the cortex (Figure 1.1; Grace, 2008; Rice et al, 2011). Dopamine regulates neuronal output by either amplifying or inhibiting signals in post-synaptic neurons (Grace and Bunney, 1984). Dopamine is released in both a tonic way, when a steady rate of action potentials lead to a more regulated and constant
release, and in a phasic way, during which action potentials fire in high frequency bursts to release dopamine in a more pulsatile fashion, as commonly seen in response to stimulation. The neurotransmitter may then be broken down by monoamine oxidases (MAO) or undergo reuptake by the presynaptic dopamine transporter (DAT). Dopamine autoreceptors also modulate further release pre-synaptically. Most striatal and substantia nigra dopamine receptors are extrasynaptic, which requires the neurotransmitter to diffuse away from the cleft before being metabolized by enzymes and eliminated. Dopamine regulation has been demonstrated as being very efficacious in the striatum compared to other areas of the brain. When a selective dopamine uptake inhibitor is administered in animals, the striatum shows a large increase in extracellular dopamine (Cragg et al, 1997). The striatum is the major input structure of the basal ganglia and highly involved in the motor deficits observed in PD (see Beaulieu and Gainetdinov, 2011 for review).
Figure 1.1 Mesocorticolimbic and nigrostriatal dopamine system pathways

Figure 1.1. Depiction of the mesocorticolimbic and nigrostriatal dopamine system in the rat brain. Dopamine neurotransmission is modulated mostly by GABAergic (γ-aminobutyric acid) and glutamatergic neurons, but also by cholinergic neurons. In the basal ganglia, dopamine is released from two nuclei; the ventral tegmental area (VTA) and the substantia nigra (SN). Dopamine neurons cell bodies originating in the ventral tegmental and sending signal to areas such as the amygdala and nucleus accumbens form the mesocorticolimbic dopamine system. In comparison, dopamine neuron cell bodies originating in the substantia nigra pars compacta and sending signal to the dorsal striatum (caudate nucleus and putamen in humans) form the nigrostriatal dopamine system (Grace, 2008; Rice et al, 2011).
Dopamine receptors exist in five different subtypes, all of which are G-protein coupled receptors (GPCR) and therefore trigger a range of intracellular signaling mechanisms through a variety of second-messenger pathways. Dopamine receptor subtypes are not uniformly distributed throughout the brain. For example, D₄ receptors are almost exclusively located in the cortex. In contrast, a large number of D₁ and D₂ receptors are located in the striatum where DRTs could potentially play a major role in the development of ICDs due to the action of dopamine agonists on D₂ receptors. Dopamine receptors are separated into two major families depending on their effect on the intracellular second messenger protein, cyclic adenosine monophosphate (cAMP). Activation of the D₁ receptor family, including dopamine receptors D₁ and D₅, is excitatory, increasing the action of cAMP. In contrast, the D₂ family, which includes D₂, D₃, and D₄ receptors, is inhibitory, decreasing the action of cAMP and inhibition of protein kinase A (PKA), leading to decreased phosphorylation of the dopamine- and cAMP-regulated phosphoprotein (DARPP) at threonine 34 (see Beaulieu et al, 2011 for review).

Nonetheless, more recent evidence shows that D₂ receptors also activate a G-protein/cAMP-independent Akt/GSK3β pathway mediated by β arrestin-2, which may dominate during prolonged, continuous stimulation of dopamine D₂ receptors (Figure 1.2; Beaulieu et al, 2011; Beaulieu et al, 2005; Beaulieu et al, 2004; Li and Gao, 2011). D₂ activation leads to G-protein uncoupling and the formation of a signaling complex composed of β arrestin-2, Akt and protein phosphatase 2A (PP2A), which dephosphorylate Akt at regulatory threonine 308 residue and negatively regulates GSK3β levels (see Golpich et al, 2015 for review). GSK3β, a target protein in the Akt/GSK3β pathway, has been involved in multiple neurological and psychiatric disorders marked by impulsivity such as drug addiction, schizophrenia and bipolar disorder in humans, as well as in hyperdopamine-dependent behaviours in animals (Beaulieu et al, 2005;
β arrestin-mediated signaling pathways have a slower onset, and a more prolonged duration than G-protein mediated GPCR signaling, and therefore can lead to long-lasting/tonic changes in dopamine concentrations or chronic agonist administration (Beaulieu et al., 2011; Beaulieu et al., 2004). Activation of this alternate GSK3β signaling pathway may contribute to DRT-induced ICDs. GSK3 is constitutively active in resting conditions and can be inhibited by phosphorylation in response to upstream signals, which then results in inhibition of the pathway (Eldar-Finkelman, 2002).
Figure 1.2 Dopamine D₂ receptor signaling pathways

Figure 1.2. Schematic of the dopamine D₂ receptor signaling pathways. The intracellular signaling pathway predominantly associated with G protein coupled D₂ receptor activation involves a reduction in cyclic adenosine monophosphate (cAMP) production and inhibition of protein kinase A (PKA), leading to decreased phosphorylation of the dopamine- and cAMP-regulated phosphoprotein (DARPP) at threonine 34. However, D₂ receptors also signal through a G-protein/cAMP-independent Akt/GSK3β pathway mediated by β arrestin-2 (Beaulieu et al, 2011; Beaulieu et al, 2005; Li et al, 2011). D₂ activation leads to inhibition of Akt1 phosphorylation by β arrestin-2 through protein phosphatase 2A (PP2A), leading to elevation in GSK3β levels.
Although a drug’s affinity refers to the degree to which it binds to a receptor, its intrinsic efficacy is usually perceived as its ability to promote a biological response (Galandrin et al, 2007). Accordingly, once activated, GPCR receptors initiate a cascade of complex intracellular downstream signals and adopt various active conformations. The capacity of an agonist drug to engage one signal transduction pathway over another by pleiotropic receptors is termed “biased agonism”. This concept suggests that biased agonist drugs can trigger a specific receptor conformation and selectively activate distinct intracellular mechanisms. The specificity provided by biased agonism can therefore produce particular or even unexpected effects of drugs (see Reiter et al, 2012 for review). Activation of β arrestin-2 in the alternate cAMP-independent pathway following activation of the D2 receptors, by interacting with the target protein GSK3β, could affect drug efficacy, as well as facilitate integration of extracellular factors and determine further molecular responses (Kenakin, 2002; Xiao et al, 2007). However, if ropinirole were acting as a biased agonist, we would expect a consistent activation of this alternate signaling cascade, a process that does not appear to be the case with ropinirole (see Andresen, 2011 for review). It was proposed that inherent properties of drugs within the same intracellular context can independently trigger various molecular signaling pathways, regardless of activity of other proteins (Galandrin et al, 2007). Also, activation of downstream kinases by GPCRs, can phosphorylate other GPCRs that are in an active state such as when activated by an agonist drug, a process termed “heterologous desensitization”, and trigger activation of independent signaling mechanisms (Benovic et al, 1986a; Benovic et al, 1986b; Lee and Fraser, 1993). For example, β arrestins activity can initiate desensitization processes in other pathways and therefore, activate unique signaling pathways (see Andresen, 2011; Ferguson et al, 1996). From this perspective, chronic ropinirole could therefore be involved in the development of DRT-induced ICDs by triggering
activation of the Akt/GSK3β pathway and β arrestin kinases and by desensitizing the cAMP/PKA pathway. Understanding the contribution of this alternate pathway could be important in understanding the development of these devastating side-effects.

As mentioned, disruption in the basal ganglia dopamine system is believed to be responsible for the motor disturbances seen in PD. Figure 1.3 depicts the basic organization of the basal ganglia direct and indirect pathway affected in Parkinson’s Disease. In this model, the basal ganglia forms two major pathways to the cortex from the substantia nigra; the direct and indirect pathways, which are kept in balance by dopamine transmission. In healthy individuals, the substantia nigra sends dopamine to GABAergic medium spiny neurons (MSNs) of the striatum. These direct pathway neurons primarily express excitatory D1 receptors, as well as substance P and dynorphin. These neurons synapse mono-synaptically on to the major output nuclei of the basal ganglia composed of the medial globus pallidum (GPi) and the substantia nigra pars reticulata (SNr). Inhibition of these nuclei leads to disinhibition of the ventrolateral thalamus, which then sends excitatory glutamate to the cortex, facilitating movement. In contrast, MSNs within the indirect pathway principally express inhibitory D2 receptors, as well as the peptide encephalin. This neuronal population synapses on to the lateral globus pallidum (GPe), which sends inhibitory GABAergic projections to the subthalamic nucleus (STN). Activation of the indirect pathway MSNs therefore ultimately disinhibits the STN, which sends excitatory glutamate to inhibitory neurons of the output nuclei (GPi/SNr). The resulting suppression of activity within the thalamus therefore exerts an inhibitory effect on motor output. In PD, depletion of dopamine disrupt this balance between the two systems, leading to excessive activity of the STN and output nuclei and exaggerated inhibition of the thalamic and brainstem functions, causing the characteristic poorness of movement seen in PD (see Blandini et al, 2000; Lanciego et al, 2012;
Obeso et al, 2002 for review). Conversely, too much dopamine, such as with the addition of L-DOPA treatment in a system that has lost its capacity to modulate extrasynaptic dopamine levels, could explain the abnormal and excessive movements observed in patients with dyskinesia (Grace, 2008).
Figure 1.3 Basal ganglia direct and indirect dopamine pathways

Figure 1.3. Organization of the basal ganglia direct and indirect pathway affected in Parkinson’s Disease. (A) In healthy individuals, the substantia nigra sends dopamine to GABAergic medium spiny neurons (MSNs) of the striatum. These direct pathway neurons primarily express excitatory D₁ receptors and synapse mono-synaptically on to the output nuclei of the basal ganglia composed of the medial globus pallidum (GPi) and the substantia nigra pars reticulata (SNr). Inhibition of these nuclei leads to disinhibition of the ventrolateral thalamus, which then sends excitatory glutamate to the cortex, facilitating movement. In contrast, MSNs within the indirect pathway principally express inhibitory D₂ receptors. This neuronal population synapses on to the lateral globus pallidum (GPe), which sends inhibitory GABAergic projections to the subthalamic nucleus (STN). Activation of the indirect pathway MSNs disinhibits the STN, which sends excitatory glutamate to inhibitory neurons of the output nuclei (GPi/SNr). The resulting suppression of activity within the thalamus exerts an inhibitory effect on motor output. In PD, depletion of dopamine disrupt this balance between the two systems, leading to excessive activity of the STN and output nuclei and exaggerated inhibition of the thalamic and brainstem functions, causing the characteristic poorness of movement seen in PD. (B) A more complex depiction of the basal ganglia involving the input from other neurotransmitters such as norepinephrine, acetylcholine, and serotonin (see Blandini et al., 2000; Lanciego et al., 2012; Obeso et al., 2002 for review). Blue arrows: dopamine modulation, Red arrows: inhibitory GABA neurons, Green arrows: excitatory glutamate neurons, Yellow arrows: serotonin neurons, Purple arrows: norepinephrine neurons, Teal dots: Acetylcholine neurons, Red dots: GABA neurons. SNc: substantia nigra pars compacta, GPe: globus pallidus externalis, STN: subthalamic nucleus, GPi: globus pallidus internalis, SNr: substantia nigra pars reticulata.
These two pathways in the basal ganglia have also been suggested to have “GO/NO-GO” properties in decision making; activation of the direct pathway would be responsible for action selection, whereas the indirect pathway would control response inhibition. From a decision-making perspective, pauses in dopamine stimulation would be needed in order to learn from negative feedback, such as when an expected reward fails to arrive (Bayer et al., 2007). Therefore, constant stimulation of D2 receptors with DRTs would impair PD patients’ ability to disengage following a negative outcome, while leaving intact learning from positive reinforcement, and this may theoretically contribute to the development of ICDs (Cools et al., 2007; Frank et al., 2004). However, this model is complicated by multiple factors. Firstly, about 15% of the GABAergic neurons in the dorsal striatum co-express D1 and D2 receptors, such that the two pathways are not completely segregated. Other neurotransmitters such as serotonin and norepinephrine also innervate the basal ganglia, and MSNs within the dorsal striatum are significantly modulated by cholinergic interneurons. A complete understanding of the role of striatal MSNs in decision making therefore needs to account for the actions of these other signaling systems. A proportion of the SNc neurons also project to the limbic, ventral striatum (i.e. Haleem, 2015; Huot and Brotchie, 2011), and could therefore alter decision making through this nucleus. In addition, denervation of dopamine in the dorsal striatum in PD leads to expression of D3 receptors on D1-expressing neurons of the direct pathway, which complicates understanding the action of DRT drugs (Bordet et al., 1997). There is therefore much that we do not understand regarding the specific mechanisms and neural pathways that contribute to the development of ICDs following DRT, and the impact of different disease states on these neurobiological processes.
1.3 Impulse control disorders in response to dopamine agonist treatment

ICDs have been increasingly recognized in PD patients, but also in patients for which dopamine depletion is not responsible for the disease such as in restless leg syndrome and fibromyalgia (Holman, 2009; Quickfall et al, 2007; Tippmann-Peikert et al, 2007). The type of ICDs developed in relation to initiation of DRTs are various and include pathological gambling, hypersexuality, compulsive shopping, binge eating, punding and excessive hobbying. As for other addiction disorders, all these ICDs are associated with an element of maladaptive preoccupation or compulsivity towards the behaviour, repeated engagement, inability to resist the urge to engage in the behaviour, and increased irritability when trying to reduce the frequency of engagement. Also, these ICDs lead to substantial distress and strain on relationships and impairment in everyday functioning, all of which are reminiscent to behaviours observed in drug addiction (see Voon et al, 2009). In contrast to ICDs, patients on L-DOPA medication are more likely to develop a syndrome known as dopamine dysregulation syndrome (DDS) defined as a compulsive use of the drug at a dose well above what is required to control the motor symptoms, even if it increases dyskinesia symptoms, similar to the disregard to consequences and behaviours observed in substance use disorder (Cantello, 2000; Giovannoni et al, 2000). Differences between men and women are also observed in the manifestation of these side-effects. For example, women are more likely to develop compulsive shopping and binge eating, whereas men are more likely to develop pathological gambling and hypersexuality (Weintraub et al, 2010). Men are also more likely to develop DDS with L-DOPA treatment (i.e. Ceravolo et al, 2010; Lawrence et al, 2003).

The mechanism that explains the development of these side-effects is still unknown, but the likelihood of developing ICDs as opposed to DDS speaks of the difference in action between
the dopamine precursor L-DOPA as opposed to preferential dopamine D_{2/3} agonist drugs. For example, although there is no difference in the proportion of patients who develop ICDs following ropinirole (15.5%) or pramipexole (17.7%), this proportion was only 6.9% in those taking other drugs (see Voon et al, 2009). A study also showed that at least 13.6% of PD patients suffer from one or more ICDs following DRT in the United State and Canada (Weintraub et al, 2010), while another implied that this number can even reach up to 25% (Hassan et al, 2011).

The risk factors for developing ICDs may be slightly distinct depending on the specific ICD. In general, a young age or young age at onset of PD, male sex, having a past or family history of alcohol or drug use, elevated score on novelty seeking, being unmarried, or smoking have all been suggested as risk factors for developing ICDs (Evans et al, 2005; Voon et al, 2007; Weintraub et al, 2015). Reports are mixed about whether higher doses of DRT increase the risk of developing ICDs (see Weintraub et al, 2015 for discussion). Nonetheless, a more rigid and introverted personality was also observed in individuals prior to developing PD, the PD personality, which suggests that a tendency towards risk may not be solely responsible (Dagher et al, 2009; Todes and Lees, 1985). In this dissertation, we focused on the development of gambling behaviours, which account for about 5-7% of all ICDs in PD patients, and compares to 0.25% in controls (Avanzi et al, 2006; Voon et al, 2017).

Pathological gambling has recently been reclassified in the Diagnostic and Statistical manual-5 (DSM-V) from the “Impulse Control Disorders” category to that of “Addiction and Related Disorder”, confirming its nature as a behavioural addiction. Also, GD and drug addiction appear to share clinical and neurobiological characteristics (Potenza, 2014). In fact, just like individuals suffering from substance addiction, individuals with GD show compulsive
engagement in the behaviour, which leads to considerable distress, and even withdrawal symptoms when individuals try to abstain (Petry, 2006; Potenza, 2006). Just like in substance addiction, the dopamine system appears to play a role in the phenomenon of GD. This evidence is supported by the development of GD in individuals with PD who take DRTs that primarily act on the D_{2/3} receptors, such as pramipexole and ropinirole (Ambermoon et al, 2011; Weintraub et al, 2006a; Weintraub et al, 2006b).

1.4 The dopamine system and DRT-induced ICDs in PD

As mentioned, loss of dopamine neurons in the substantia nigra (SNc) and consequential loss of dopamine terminals in the dorsal striatum are involved in the pathology of PD. In pre-symptomatic PD, the loss of dopaminergic transmission from the SNc is believed to be accompanied by an increase in efficacy of the remaining dopamine neurons such that they release more dopamine in the striatum, thereby compensating for damage to the system and masking early symptoms of the disease (Zigmond et al, 1990). In early stages of symptomatic PD, a significant loss of about 50-80% of dopamine neurons and depletion of synaptic vesicles due to increased dopamine turnover has already occurred (Wilson et al, 1996). The severe loss of dopaminergic terminals in PD also decreases phasic, as well as tonic, dopamine release. DRT treatment is believed to replenish dopamine stores in the dorsal striatum, where the damage is more severe. Given the involvement of the dopaminergic system in the pathology of PD and the development of ICDs in response to DRTs, the “overdose” hypothesis has been suggested in which DRTs would restore functioning in the basal ganglia and improve behaviours in PD patients, but overwhelms the mostly spared mesolimbic reward system, creating the behavioural side-effects observed (Cools, 2006; Kish et al, 1988). However, although the specific
involvement of the various dopaminergic systems in contributing to the development of DRT-induced ICDs has been the topic of multiple studies, the mechanism by which they occur is still unknown. In addition, various studies in humans and animals suggest that depletion of dopamine as seen in PD is not necessary for ICDs such as PG to appear, but instead that DRTs alone can precipitate the development of ICDs (Rokosik and Napier, 2012; Tremblay et al, 2016; Voon et al, 2011c).

Further complicating understanding of the development of GD in PD following DRT treatment, studies begin to highlight differences between GD and stimulant drug addiction. For example, while acute drug taking results in increased activity of the ventral striatum and sensitization of the dopaminergic system, long-term substance addiction is associated with a reduction in D_{2/3} receptor levels and blunted activity in this area in humans (Martinez et al, 2004; Volkow et al, 2007). Although a functional magnetic resonance imaging (fMRI) study showed decreased activity in the mesolimbic system in response to monetary reward in individuals with GD (Reuter et al, 2005), a number of studies did not find evidence of lower dopamine D_{2/3} receptor levels in individuals with GD compared to healthy control subjects at baseline in this area (i.e. Boileau et al, 2013; Joutsa et al, 2012). In PD patients, one study has reported no difference in D_{2/3} receptors levels between those with or without ICDs (Payer et al, 2015b), whereas another found an increase in dopamine release in PD with GD (Steeves et al, 2009). Looking beyond the ventral striatum, evidence supports a role for the dorsal striatum in the transition to more habitual and compulsive behaviour in addiction (Everitt and Robbins, 2005). A recent study has shown elevated dopamine release in the dorsal striatum in pathological gamblers, which was correlated with increased dopamine in the ventral striatum and also associated with gambling severity (Boileau et al, 2014). Increased activity in the dorsal striatum
has also been involved in anticipation of monetary rewards in GD patients, suggesting a potential role for this area in GD and potentially also in DRT-induced ICDs (van Holst et al., 2012b).

Interestingly, the lateral orbitofrontal cortex (OFC) has strong connections with the dorsal striatum and is thought to be involved in updating the subjective value of rewards. As mentioned above, dopamine agonists such as ropinirole and pramipexole, have a high affinity for the D\textsubscript{2} receptor family. Therefore, persistent stimulation of D\textsubscript{2} receptors in the basal ganglia indirect pathway was hypothesized to abolish the decrease in tonic dopamine release in response to negative outcomes and impair negative feedback learning in PD with ICD (Dagher et al., 2009; Frank et al., 2004). In an fMRI study in PD patients without ICDs, increased lateral OFC activity was observed during negative feedback following pramipexole, suggesting that this drug may impair reward processing in the OFC (van Eimeren et al., 2009). These studies therefore suggest a potential important role for the dorsal route including the OFC and dopaminergic innervation to the striatum, rather than ventral route, in subjective value-judgments that modulate choice bias under uncertainty.

However, the effect of stimulating D\textsubscript{2} receptors is complex. These receptors are inhibitory, and can be autoreceptors located on pre-synaptic dopamine neurons, as well as post-synaptic receptors located on inhibitory interneurons and medium spiny neurons in the striatum (Ford, 2014; Seamans and Yang, 2004). Also, stimulation of autoreceptors in the ventral striatum is believed to reduce the excitatory influence from the prefrontal cortex (Grace et al., 2007). Therefore, understanding the contribution of specific pathways such as the nigrostriatal pathway, which may modulate the influence of the OFC, as well as the relative involvement of dopamine D\textsubscript{2} receptors in response to dopamine D\textsubscript{2/3} agonists, is urgently needed. Specifically, understanding the impact of chronically activating and deactivating the nigrostriatal pathway on
decision making under uncertainty could therefore help us understand the mechanism by which dopamine D2/D3 agonists drug such as ropinirole can induce ICDs such as GD.

1.5 Animal models of decision making

Animal models that capture changes in impulse control or risky decision making, and that are sensitive to chronic DRT administration, could make a significant contribution in understanding the mechanism by which DRTs may induce the development of ICDs. The tighter degree of experimental control possible in animal studies makes it easier to determine whether premorbid behavioural traits contribute to a maladaptive drug response. Such models could also help elucidate the biological mechanism underlying DRT-induced changes in decision making, and contribute to the search for adjunctive drug treatments that would offset the psychiatric complications of DRTs while preserving the control of motor symptoms. Although some differences in neurobiology may occur between rodents, non-human primate and humans, many structures and connectivity appear to be conserved between species. As an example, the striatum, which can be distinguished into the caudate and putamen in humans and non-human primates, is undistinguishable in rodents. However, there is a large overlap in connectivity such that the corticostriatal integration in neural pathways between these two species is mostly conserved (Haber and Knutson, 2010; Heilbronner et al, 2016). In addition, it has been shown that humans, non-human primate and rodents are all sensitive to reward size and probability, however with subtle differences. Although humans and non-human primates are ambiguity-averse and prefer options with a known probability, there are greater individual differences in risk preferences in rodents that may depend on the task used. Nonetheless, all species have a tendency to base future decisions on previous outcomes and follow a win-stay/lose-shift choice
pattern. Also like humans, rodents were shown be able to learn to choose long-term advantageous options, making them a valid choice to study risky decision-making behaviours (see Heilbronner, 2017 for review). Our laboratory has developed a number of rat paradigms that measure different aspects of decision-making under risk and uncertainty, with the goal of modeling cognitive behavioural factors that capture vulnerability to gambling disorder. In this dissertation, we tested the effect of chronic D2/3 ropinirole on two tasks that assess various aspects of decision-making; the rodent Betting task (rBT) and the rat Gambling task (rGT).

1.5.1 The Rodent Betting Task

Similar to individuals with GD, PD patients show deficits in a variety of decision-making tasks. Individuals with problem gambling make higher rates of risky choices even when the probability of reward decreases on a probability discounting task (Madden et al, 2009), and PD patients on dopamine replacement therapy (DRT; including DRT and levodopa) show increased risky decisions on probabilistic tasks such as the Game of Dice (Brandt et al, 2015; Labudda et al, 2010). Recent work using animals suggests that repeated administration of the D2/3 agonist pramipexole increases choice of the risky option on a probability discounting task in both healthy rats and in a rat model of PD (Rokosik et al, 2012), further suggesting that DRT alone is primarily responsible for the behavioural changes observed. Psychobiological accounts of GD posit that strong cognitive distortions in decision-making can contribute to the etiology and severity of GD (Clark, 2010).

In human gambling, variation in the size of a possible win or loss alters the willingness to gamble such that individuals are less prone to make a risky choice if the size of the wager is larger as opposed to smaller even when the odds of winning remains the same, an effect that has
been termed “escalation of commitment” (see Winstanley and Clark, 2016a for review). This aversion towards risky decisions when the bet size increases, even though the expectancy is the same in both options may therefore be considered mathematically irrational (Tversky and Kahneman, 1974).

Somewhat modeling the escalation of commitment phenomenon, the rBT was developed as one of a series of tasks aiming to capture non-normative decision-making biases evident in human decision making under uncertainty (see Cocker and Winstanley, 2015 for discussion). A task schematic describing the rBT is shown in Figure 2.2. In short, this task captures the degree to which animals tolerate risk and change their preference for an uncertain option based on the magnitude of the bet size in play when the uncertainty is maximal- i.e. when the probability of winning or losing is 50% (Cocker et al, 2012a). In the rBT, rats choose between two options matched for expected value. As such, there is no net advantage in choosing one option over the other. A response on the safe lever leads to guaranteed delivery of a small reward, whereas pressing the uncertain lever leads to double the safe reward or nothing on a 50:50 basis. By extensively training the animals on the task to the point where they are familiar with the contingencies in play, the rBT allows us to look at decision-making under uncertainty without the confound of differences in learning rates, and to investigate rats’ decision-making preferences when there is no real risk in choosing the uncertain option.

On the rBT, a significant proportion of rats decrease their choice of the uncertain option when the bet size increases, akin to the escalation of commitment phenomenon observed in humans (Cocker et al, 2012a; Tremblay et al, 2014). Consistent with the idea that the dorsal striatum may be involved in GD, D2/3 receptor expression in this area was also associated with the manifestation of this irrational choice bias, such that rats who decreased their choice of the
uncertain option as the bet size increased had lower expression of D$_{2/3}$ in this area (Cocker et al., 2012a). Therefore, D$_{2/3}$ receptor level in the dorsal striatum appears to be associated with this marker of addiction vulnerability. Furthermore, inactivating the lateral OFC on-task increases choice of uncertainty in risk-averse rats, diminishing non-normative bias (Barrus et al., 2016a). In contrast, lesions to the basolateral amygdala, which is strongly interconnected with the mesolimbic dopamine system, has no effect on this decision-making bias (Tremblay et al., 2014).

The rBT is similar to the standard trial in the “gain” condition of a gambling-like task used recently in humans in which, like in the rBT, there was no real risk in choosing either the safe or uncertain option. On this task, PD patients with ICDs showed greater preference for the uncertain option compared to PD patients without an ICD (Voon et al., 2011a). Collectively, these data support the potential role of the dorsal, rather than ventral, striatal signaling in GD, and potentially in DRT-induced ICDs. Assessing the effect of dopamine D$_{2/3}$ agonists on the rBT could therefore be informative with regards to the effect of these drugs on subjective preference for uncertainty and gambling-like behaviours.

1.5.2 The Rat Gambling Task

Although originally developed to model “real-world” decision making, in which each option is associated with the chances of both good and bad outcomes, the Iowa Gambling Task (IGT) has been increasingly used as a neuropsychological test to assess cognition in relation to gambling behaviour. This task uses rewards and punishments to assess risky and ambiguous decision making in humans (Bechara et al., 1994). In this task, participants chose a card from one of four decks, each card having a different monetary value. The goal of the task is to accrue as much money as possible, without previously knowing the contingencies associated with each deck of cards. However, the cards in each deck are not equivalent. In decks A and B, the cards
deliver larger amounts of monetary reward, but also higher penalties. Decks C and D have lower rewards, but also lower penalties such that the optimal strategy is to favour decks C and D. Ultimately, consistent choices from the tempting “high-risk high-reward” decks A and B lead to monetary losses over successive trials, whereas choosing from decks C and D leads to monetary gain.

The rat gambling task (rGT) was based on the IGT used clinically. A task schematic describing the rBT is shown in Figure 2.3. As in the IGT, the rGT requires rats to balance the probability of receiving smaller or larger rewards against the chances of receiving time-outs in order to maximize sugar pellet profits (Zeeb et al, 2009). Similar to the IGT, the optimal strategy is to avoid the tempting high-risk high-reward options, and instead favour those associated with smaller per-trial gains but also lower penalties. A growing body of literature suggests that performance of the rGT taps into similar cognitive processes, and is dependent on comparable brain circuitry, as the IGT used in humans (Young et al, 2011; Zeeb et al, 2009; Zeeb and Winstanley, 2011). Elevated preference for the disadvantageous risky options is observed in numerous patient populations, including (but not exclusively) those with disorders hallmarked by high impulsivity such as gambling disorder (Alvarez-Moya et al, 2011; Cavedini et al, 2002; Kertzman et al, 2011), bipolar disorder (Clark et al, 2001), substance use disorder (Bechara and Damasio, 2002) and suicidality (Jollant et al, 2005). However, whether PD patients with ICDs perform worse on the IGT has not been convincingly demonstrated. Although selective and stable deficits in IGT performance were reported in a patient with early-onset PD who developed GD following treatment with L-DOPA and then DRT (Pignatti et al, 2012), a recent study reported only trend-level increases in risky choice in a cohort of PD patients with ICDs, despite higher scores on the Barratt Impulsivity Scale (Bentivoglio et al,
Since the rBT and rGT may tap into different cognitions, and possibly different neurobiological pathways (see Cocker et al, 2015 for review), it would be useful to compare the effect of DRT on decision-making on the rGT and the rBT.

Studies of drug addiction have accorded much importance to the influence of cues in the pathology (Obrien et al, 1992). Similar to the significance of cues in drug addiction, cues associated with gambling have been implicated in the transition from recreational gambling to disordered gambling (Grant and Bowling, 2015; van Holst et al, 2012a). In animals, cues that predict rewards increase the release of dopamine (Schultz, 1998). However, reward-paired cues are not inherent in the standard rGT. Our laboratory has shown that adding complex audiovisual cues associated with wins in the rGT, a paradigm now known as the cued rGT, increases the proportion of rats preferring the disadvantageous options, and most specifically, the option with maximal uncertainty, P3, rewarded on 50% trials (Barrus and Winstanley, 2016b). Previous studies have shown that repeated exposure to stimuli that predict uncertain outcomes can sensitize the dopamine system (Singer et al, 2012; Zack et al, 2014). It is therefore possible that exposure to cues associated with wins in the cued rGT may sensitize the dopamine system and potentially trigger an effect of chronic ropinirole on this task. Individual differences in cue-induced maladaptive decision-making on the cued rGT could also reveal a vulnerability to the effect of ropinirole. Therefore, we also compared the effect of chronic ropinirole on the cued rGT to that on the standard rGT in order to evaluate the potential influence of cues in DRT-induced ICDs.

1.6 The use of DREADDs to manipulate dopamine in the nigrostriatal pathway

While genetic manipulations and invasive techniques are unfeasible in human subjects,
recent chemogenetic technological advances now make it possible to causally manipulate
specific neural and molecular pathways in rodent models. Recently, advantages brought about
by Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), allow
investigation of the putative role of specific pathways involved in the onset of DRT-induced
ICDs. DREADDs are engineered receptors that are mutants of the human muscarinic GPCR
receptors. They are inactive and insensitive to the endogenous acetylcholine neurotransmitter,
but were designed to be activated by the otherwise inert exogenous ligand clozapine-n-oxide
(CNO) (Urban and Roth, 2015). DREADDs are introduced into neural tissue by infusion of
vector viruses such as adeno-associated-virus (AAV) into a target area, after which the synthetic
receptors are then expressed both on cell bodies and axons (Mahler et al, 2014; Zhu and Roth,
2014). More specifically targeting neurotransmitter systems can be achieved by using transgenic
animals and cre-dependent DREADDs. For example, translation and transcription of cre-
dependent DREADDs only occurs in the presence of cre recombinase, an enzyme that is inserted
into transgenic lines to control transgene expression. Our laboratory now breeds transgenic rats
engineered to selectively express cre recombinase in neurons that synthesize tyrosine
hydroxylase i.e. dopaminergic and noradrenergic neurons (TH:cre rats). Delivering cre-
dependent DREADDs into the SNc of TH:cre positive rats via viral-mediated gene transfer will
result in selective DREADDs expression in dopaminergic afferents that can then be activated or
suppressed by systemic administration of CNO in transgene positive rats. In this case, transgene
negative rats that do not express cre recombinase, can act as control subjects (Smith et al, 2016).
CNO is metabolized into clozapine and N-desmethylclozapine in rodents, humans and non-
human primates (Chang et al, 1998; MacLaren et al, 2016; Raper et al, 2017). For this reason,
experiments using TH:cre negative rats as control in which all rats, transgenic positive and
negative, are injected with a dose of CNO is a major advantage over those using a vehicle injection as control.

It is believed that about 66% of cells in the targeted brain area will express DREADDs receptors following AAV vector infusion, so not all cells are affected (Gremel and Costa, 2013; Smith et al, 2016). Therefore, in contrast to microinfusion techniques in which inhibition is more complete, the DREADDs technique reduces, in the case of inhibitory DREADDs, rather than completely eliminates the contribution of a targeted brain area. Similarly, excitatory DREADDs’ activation will facilitate cell firing rather than causing action potentials (Smith et al, 2016). This may be an advantage, considering that it is more similar to the action of endogenous GPCR activations by neurotransmitters in the brain. Also, it appears that excitatory DREADDs may be more efficient than inhibitory DREADDs. Studies have shown that after AAV infusion, most cells recorded in the locus coeruleus fired following systemic CNO administration, and that excitation was more than double that at baseline (Vazey and Aston-Jones, 2014a). However, only close to 80% of cells would be affected in the case of inhibitory DREADDs with inhibition reaching about 60% of their baseline activation (Chang et al, 2015). These authors also determined that the onset latency of CNO following IP injection occurs on average within 12 minutes and can last for more than 70 minutes. Therefore, the relatively long half-life of CNO and its more naturalistic approach may be very useful in trying to understand the contribution of specific pathways involved in DRT-induced ICDs in animal behavioural studies.

Multiple studies have demonstrated the involvement of various brain areas in behaviours using DREADDs. For example, a study showed the involvement of the medial prefrontal cortex in the facilitation of associative learning (Yau and McNally, 2015), while another has shown that inhibition of the ventral pallidum, an area rich in D3 receptors, impairs operant learning (Chang
et al, 2015). In relation to decision-making, studies have shown the importance of the OFC in evaluating expected outcomes in a signaled-probability task. Inhibition of this brain area with DREADDs impaired animals’ ability to use the relation between a signal and outcome probability to guide decision-making behaviours (Ward et al, 2015). The use of chemogenetic systems such as DREADDs to develop animal models of diseases is also increasing (see Whissell et al, 2016). In this dissertation, we therefore attempt to capitalize on the advantages brought about by DREADDs, to investigate the putative role of the nigrostriatal pathway in the onset of gambling-like behaviours induced by initiation of DRTs.

1.7 Summary

In summary, DRTs such as ropinirole and pramipexole have been developed and used as highly effective treatment for the motor symptoms of PD. However, a significant minority of patients develops devastating ICDs such as GD following initiation of these drugs. These ICDs have also been observed in other patient populations for which they are prescribed, including restless leg syndrome and fibromyalgia (Holman, 2009; Quickfall et al, 2007; Tippmann-Peikert et al, 2007; Voon et al, 2011c). Whether prior tendency towards risky behaviours plays a role in conferring vulnerability is unclear (Voon et al, 2011d; Voon et al, 2007). Nonetheless, understanding the mechanism by which DRT triggers GD, which could help us develop strategies to mitigate these ICDs, would be of significant benefit in keeping the positive outcomes on motor symptoms these drugs provide to PD patients.

Ropinirole and pramipexole are dopamine agonists at the D$_{2/3}$ receptors. Recent evidence suggests that continuous activation of D$_2$ receptors with dopamine D$_{2/3}$ agonists may trigger activation of an alternative intracellular cascade through the Akt/GSK3β pathway ultimately
leading to elevation of the target kinase GSK3β. Elevation of this kinase may contribute to the development of ICDs in PD patients due to its involvement in multiple neurological and psychiatric disorders marked by impulsivity in humans and animals (Beaulieu et al, 2011; Beaulieu et al, 2005; Beaulieu et al, 2004; Li et al, 2011; Peterson et al, 2015b). Understanding the involvement of this kinase in the development of ICDs could enable us to develop strategies to mitigate these unwanted side-effects.

Loss of dopamine neurons in the substantia nigra (SNc) and consequential loss of dopamine terminals in the dorsal striatum are involved in the pathology of PD. The “overdose” hypothesis has been suggested in which DRTs would restore functioning in the basal ganglia and improve motor behaviours in PD patients, but overwhelm the mostly spared mesolimbic reward system, creating ICDs (Cools, 2006; Kish et al, 1988). Many studies have investigated the specific involvement of the various dopaminergic systems in contributing to the development of ICDs following initiation of DRTs, but the mechanism by which they occur is still unknown. Furthermore, evidence supports a role for the dorsal striatum and its connection with the OFC in GD. Therefore, the dorsal route including the OFC and dopaminergic innervation to the striatum, rather than ventral route, may contribute to GD and also potentially to DRT-induced ICDs (Dagher et al, 2009; Frank et al, 2004; van Eimeren et al, 2009). Specifically, understanding the implication of the nigrostriatal pathway on the development of ICDs such as GD could help us understand the mechanism by which dopamine D2/3 agonists drugs such as ropinirole can induce ICDs such as GD.
1.8 Objectives

The main objective of experiment 1 and 2 in Chapter 3 was to demonstrate that, similar to what is observed in humans, chronic administration of the dopamine D_{2/3} agonist ropinirole increases preference for uncertainty in a significant proportion of rats performing the rodent Betting task (rBT), a task designed to capture the degree to which animals’ preference for a safe or uncertain option may change as a function of the bet size in play. It also showed that this change in decision making occurred regardless of dopamine depletion in a dorsolateral striatal 6-OHDA lesion model of PD, or animals’ baseline preference for the uncertain or safe option.

In the first experiment in Chapter 4, we showed that, in contrast to the changes observed on the rBT, chronic ropinirole did not alter decision-making under risk on a rat analog of the Iowa Gambling task (IGT), the rat Gambling task (rGT). Given that cues are strongly implicated in addiction gambling disorders, we then aimed to test whether adding cues associated with wins on the rGT could trigger a change in decision-making on this task following chronic ropinirole.

The canonical intracellular mechanism engaged following activation of the GPCR D_{2} receptors leads to decreased action of cAMP. However, under prolonged activation such as with administration of dopamine agonists, a cAMP-independent Akt/GSK3β pathway may be activated, leading to increased GSK3β protein expression, a protein that has been involved in various disorders marked by impulsivity. Therefore in the experiments in Chapter 5, we aimed to determine if administration of a selective GSK3β inhibitor can reduce or block the increase in choice of uncertainty observed following chronic ropinirole administration on the rBT. We also investigated ex vivo levels of key proteins in dopaminergic signaling pathways using western blot.
Finally, in Chapter 6, we proposed to investigate the impact of manipulating DA tone in the nigrostriatal pathway in the response to ropinirole using DREADDs. The overdose hypothesis in DRT-induced ICDs stipulates that DRTs restore functioning in the basal ganglia where the damage is more pronounced, and therefore improves behaviours in PD patients, but overwhelm the mostly spared mesolimbic reward system, creating the behavioural side-effects observed. However, the neurobiology underlying the development of ICDs in PD in response to DRT is unclear, and research on gambling disorders suggest that the dorsal route in the basal ganglia, rather than the ventral route may be involved. We therefore investigated the effect of excitation of the nigrostriatal pathway in potentially mimicking the effect of ropinirole on the rBT. In comparison, we also explored the effect of inhibiting this pathway in potentiating the increase in uncertain choices created by ropinirole on the rBT.
Chapter 2: General materials and methods

2.1 Subjects

Subjects in Chapters 3, 4 and 5 were male Long Evans rats (Charles River Laboratories, St. Constant, Canada), aged around post-natal day 60 and weighing between 250 - 275 g at the start of the experiments. In Chapter 6, subjects were TH:cre transgenic male and female rats, bred in-house (Long-Evans-Tg (TH-Cre) 3.1Deis, RRRC #00659; Rat Resource and Research Centre, Columbia, MO; wildtype females obtained from Charles River, St. Constant, Canada). All rats were housed in a climate-controlled colony room on a reverse 12 hours light-dark cycle (lights off 08.00; temperature 21°C). Water was always available ad libitum. Subjects incoming from Charles River were pair-housed from the time of their arrival. In-house bred animals were pair-housed once they had reached 250 – 275 g for the males, or tripled-housed once they had reached 200 g for the females. All rats were free fed for at least a week, during which the experimenter handled them daily. Rats were then food restricted to 85% of their free-feeding weight and maintained on standard rat chow (males: 14 g; females: 9 g) per day plus any sugar pellets earned on task (~5 g per day). Behavioural testing began one week following the start of food restriction. Rats were trained between 7:30 am and 6:00 pm five to six days a week. All housing conditions and testing were in accordance with the Canadian Council on Animal Care, and the University of British Columbia Animal Care Committee approved all experimental procedures prior to the beginning of the study.

2.2 Behavioural apparatus

Behavioural testing took place in 28 standard five-hole operant chambers from Med Associates Inc, Vermont, USA (Figure 2.1). Each was individually housed in a ventilated and
sound-attenuating cabinet. Each chamber featured an array of five response holes on one side, and a food magazine, which was positioned midline on the wall opposite to the response hole array. Each response hole, as well as the food magazine, was equipped with a stimulus light at the back and a horizontal infra-red beam passing across to detect a nose-poke. The response holes and the food magazine were positioned two cm above a bar floor. The food magazine delivered sucrose pellets rewards (45 mg; Bioserv, New Jersey) from a connected pellet dispenser fitted outside of the chamber. Each chamber was also furnished with a house light to allow for illumination and was controlled by software written in Med PC by Catharine A. Winstanley (CAW) running on an IBM-compatible computer.
Figure 2.1 Standard five-hole operant chamber

Figure 2.1. The standard five-hole operant chamber used for the rBT and rGT. (A) Side view of a MedAssociates five-hole box. These chambers are modular and can be configured for multiple behavioural paradigms and manipulations, hence the arm assembly for drug self-administration/microdialysis and additional levers visible. (B) A close-up of the standard five-hole array showing stimulus light location.
2.3 Behavioural testing

On training days, rats were transported to the testing room in their home cage prior to being placed in the operant chamber. Rats were consistently placed in the same operant chamber for the duration of behavioural testing.

2.3.1 Habituation and training

All subjects were initially habituated to the testing chambers by freely accessing sucrose pellets placed in each response hole. Following two such 30 min sessions, animals were trained to nose poke in the response holes when they were illuminated using either the Five-Choice Serial Reaction Time Task (5CSRT) in rats that were to perform the Betting Task, or a revised version adapted for the rat Gambling task (rGT) in those to perform the rGT or the Cued rGT. The Betting task uses the three middle holes omitting the two most peripheral ones whereas the rGT task uses the four most peripheral holes omitting the middle hole. In the 5CSRT and the rGT Four-Choice training, a light in one of the five holes become illuminated. These illuminated stimuli were presented one at a time pseudorandomly, in sessions that consisted of either 100 trials or 30 minutes. Animals were rewarded when they performed a nose poke in the appropriate aperture within 10 sec. Rats to be trained on the rGT or the cued rGT started training on a forced-choice version of the task once accuracy in responding to the illuminated hole attained more than 80 percent, with less than 20 percent trials omitted. Trials were scored as an omission if rats failed to respond at the array within 10 sec. Once they had reached similar accuracy on the 5CSRT, rats to perform the Betting task were subsequently trained to press retractable levers for reward on a fixed ratio 1 schedule. Only one lever was presented per session. Once the animal was making > 50 lever presses per session, training was repeated on
the other lever. The order in which the levers were presented (i.e. left versus right) was counterbalanced between subjects.

2.3.2 The rodent Betting Task

The rBT—Forced-Choice. Task training began with a forced-choice version of the rBT, wherein only one of the levers was extended per trial in a pseudorandom fashion. Each lever was permanently designated as safe or uncertain and counterbalanced across rats. Each session consisted of 12 blocks of 10 trials. The bet size remained constant within each block but varied between blocks in a pseudo-random fashion which ensured 4 blocks of each bet size within a session, and not more than 2 consecutive blocks of the same bet size. Following a nose poke in the illuminated food tray, either 1, 2, or 3 lights came on in hole 2, 3, and 4 to signal the bet size in play. A nose poke in each of the response holes, in any order, was necessary to extinguish the light. Once all the stimulus lights had been turned off in this manner, a lever was inserted into the chamber. A safe lever press guaranteed delivery of the number of sugar pellets associated with the number of lights presented whereas a response on the uncertain lever resulted in twice the reward or nothing on a 50:50 basis. The expected value of both options was therefore equal. On rewarded trials, the designated number of pellets was dispensed into the food tray. Regardless of whether reward was delivered, the tray light was illuminated after a response had been made on one of the 2 levers, and a response at the food tray initiated the next trial. Failure to respond on either lever within 10 s led to the trial being scored as a choice omission. Similarly, failure to respond at all illuminated apertures within 10 s resulted in the trial being scored as a hole omission. Both errors of omission were immediately punished by a 5 s time-out period. During such time-outs, the house-light illuminated the chamber and no reward could be earned or trials initiated. Following the time-out period the tray light was illuminated, indicating
the animal could commence another trial. Animals were trained on the forced-choice version for about 10 sessions, after which they progressed to the free-choice version of the task. Sessions lasted until all 120 trials had been completed, up to a maximum duration of 30 min.

The rBT—Free-Choice. The free-choice version of the rBT was identical to the forced-choice version, except that both levers extended, allowing the animal to freely choose either the safe or uncertain lever by pressing the corresponding lever. Figure 2.2 shows the trial structure of the rBT. Again, each session consisted of 12 blocks of 10 trials. The bet size remained constant within each block but varied between blocks in a pseudo-random fashion which ensured 4 blocks of each bet size within a session, and not more than 2 consecutive blocks of the same bet size. Within each block, the probability of receiving reward following a response on the uncertain lever was constrained such that animals were guaranteed to win on no more than 50% of trials within each block, even if they chose the uncertain lever exclusively. To ensure the animal sampled from both options throughout the session and was familiar with the current contingency in play, the first 4 trials of each block were forced choice, such that only the safe (2 trials) or uncertain (2 trials) lever was presented in random order. Each session lasted for 30 minutes after which the rat was transported back to its home cage and food was distributed by the experimenter. Animals were trained 5 to 6 times per week and tested until pattern of choice was stable over 5 sessions as determined by a non-significant effect of session or session x bet size in a repeated-measures analysis of variance (ANOVA).
Figure 2.2. Schematic of the rodent betting task (rBT). Each trial began by making a nose-poke response at the illuminated food tray. The tray light was then extinguished, and 1–3 response holes were illuminated, signaling the size of the bet/wager (1–3 sugar pellets). A nose-poke response at an illuminated aperture turned off the light inside it. Once all of the aperture lights had been extinguished in this manner, two levers were presented to the rat. Selection of the uncertain lever resulted in a 50:50 chance of receiving either double the wager or nothing, whereas selection of the safe lever always led to delivery of the wager. The trial was scored as a choice omission if the rat failed to choose one of the levers within 10 s. Likewise, if the rat failed to respond at each illuminated response hole within 10 s, the trial was scored as a hole omission. Adapted with permission from Cocker and colleagues (Cocker et al., 2012b).
2.3.3 The rat Gambling Task

*The rGT—Forced-Choice.* Rats began the rGT training on a forced-choice version of the task where rats were presented with only one illuminated hole similar to training on the Four-Choice training of the rGT. This ensured that all rats sampled each of the different options and learned the contingencies associated with these options. In this task, each hole is associated with a different magnitude and probability of reward (one through four sugar pellets; P1-P4) and punishment (5-40 s time-outs). The location of the pellet choice options (P1–4) was counterbalanced across animals such that half the animals were tested on version A and half on version B. According to the hole order in the 5-hole operant chamber (left to right: 1, 2, 4, and 5), the order of pellet options in version A was P1, P4, P2, and P3, in version B was P4, P1, P3, and P2. P1 was rewarded on 90% of trials and associated with a 5 sec time-out penalty, P2 was rewarded on 80% of trials and associated with a 10 sec time-out penalty, P3 was rewarded on 50% of trials and associated with a 30 sec time-out penalty, and P4 was rewarded on 40% of trials and associated with a 40 sec time-out penalty. The position of these holes and associated probability of reward and punishment, once allocated, remained unchanged for the duration of behavioural testing. These reinforcement contingencies were designed such that the best option was P2, and consistent choice of this option led to the maximal amount of sucrose pellets over time. The larger sucrose pellet options lead to larger gains on rewarded trials, but consistent choices of these options also led to larger punishments over time and less possibility to complete as many trials and earn as much reward within the 30 min allocated for a session.

*The rGT—Full Program.* Figure 2.3 shows the trial structure of the rGT. Subjects initiated each trial by making a nose-poke response in the illuminated food magazine. This response extinguished the tray light and triggered the start of a 5 s inter-trial interval (ITI). At
the end of the ITI, holes 1, 2, 4, and 5 were illuminated for 10 s. The trial was scored as an omission if animals failed to respond within 10 s, at which point the tray-light was re-illuminated and animals could start a new trial. A response in any illuminated hole turned off all stimulus lights, and led to either onset of the tray light and delivery of reward or the start of a time-out “punishment” period. If the trial was punished, no reward was delivered and the stimulus light within the chosen hole flashed at 0.5 Hz until the punishing timeout had elapsed, at which point the tray light was illuminated. A response in the food magazine started the next trial after both reward and punishment. In parallel to the 5CSRT, premature responses made at the array during the ITI were punished by a 5 s time-out period, signaled by illumination of the house light, after which the tray light was re-illuminated and animals could start a new trial. Similar to training on the rBT, each session lasted for 30 minutes after which the rat was transported back to its home cage and food was distributed by the experimenter. Animals were trained for 5 to 6 sessions per week and tested until a statistically stable pattern of choice was observed over 5 sessions as determined by a non-significant effect of session or session x option in a repeated-measures ANOVA.
Figure 2.3. Schematic of the rodent gambling task (rGT). Each trial began with the illumination of the tray light. A nosepoke in the tray extinguished the tray light and initiated a 5-second intertrial interval (ITI), during which all lights in the chamber were off. After the ITI, stimulus lights were illuminated in apertures 1, 2, 4 and 5, each of which was associated with a different schedule of reward/punishment. If the animal nosepoked one of the apertures within 10 seconds, the animal was rewarded or punished according to the schedule associated with that aperture. The size of reward and duration of punishment for each option are indicated on the schematic; the p value in brackets beneath each of those indicates the probability of a win or loss on any given trial. On a rewarded trial, the tray light was illuminated and the requisite pellets dispensed. A response at the tray then initiated a new trial. On a punished trial, the light in the chosen aperture flashed at a frequency of 0.5 Hz for the duration of the time-out period; all other lights were extinguished. At the end of the time out, the tray light was once again illuminated and the animal could initiate a new trial. A nosepoke at an aperture during the ITI was scored as a premature response and initiated a 5-second time-out period during which the house light was illuminated. Failure to make a response at an aperture within 10 seconds of the stimulus lights being illuminated was scored as an omission; the stimulus lights were extinguished, the tray light was once again illuminated, and the animal was able to initiate a new trial. Adapted with permission from Zeeb and colleagues (Zeeb et al, 2009).
2.3.4 The cued rGT

The training and structure of the cued rGT was identical to that of the original uncued rGT except for the introduction of audiovisual cues that accompanied reward delivery on winning trials and varied in complexity across options that were present in the forced-choice and free-choice stages of the task. Figure 2.3 depicts the trial structure of the cued rGT. The complexity of win-associated cues increased as win size increased, similar to the experience of human gambling games. Previous studies showed that rats will respond for complex, variable audiovisual stimuli, and our pilot data indicated that rats respond preferentially for lights that flash at higher frequencies (Barrus et al, 2016b; Olsen and Winder, 2009). Table 2.1 shows the details for the auditory and visual cues associated with wins in the cued rGT.
Table 2.1 Stimuli used in the cued rGT

<table>
<thead>
<tr>
<th>Option</th>
<th>Cue duration</th>
<th>Auditory cues</th>
<th>Visual cues</th>
<th>Variable?</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>2 sec</td>
<td>1 tone</td>
<td>Flash H1, 2.5 Hz, 2 sec</td>
<td>No</td>
</tr>
<tr>
<td>P2</td>
<td>2 sec</td>
<td>2 tones, in sequence, 1 sec each</td>
<td>Flash H4, 2.5 Hz, 2 sec</td>
<td>No</td>
</tr>
<tr>
<td>P3</td>
<td>2 sec</td>
<td>3 tones, in sequence, 0.2 sec each</td>
<td>Flash H5, 5 Hz, 1 sec; Flash H2, H3, H4, 5 Hz, 1 sec</td>
<td>Yes, 2 patterns</td>
</tr>
<tr>
<td>P4</td>
<td>2 sec</td>
<td>6 tones, in sequence, 0.2 sec each</td>
<td>Flash H2, 5 Hz, 1 sec; Flash H1, H2, H3, H4, H5, 5 Hz, 1 sec</td>
<td>Yes, 4 patterns</td>
</tr>
</tbody>
</table>
In brief, a win on a P1 trial corresponded with a light flashing at 1 Hz in the corresponding hole, the tray light solidly illuminated, and a single tone concurrently playing. The hole and tray light during a win on a P2 trial was identical to a win on a P1 trial, but the tone sequence was composed of 2 distinct 1 s tones delivered in the same order on each trial. The pattern for win-associated cues for P3 and P4 was more complex and used 6 different tones that were previously validated as discriminative stimuli (Rogers et al, 2013; Winstanley et al, 2011). The 6 tones used were as follows: 4, 8, 10, 12, 15, and 20 kHz. Using the letters A–F to represent a different tone, the patterns for P3 were: CDEDCDEDCD; CECEDEDECE. Similarly, the patterns for P4 were: ABCDEFEDCB; BCDCDEEDEF; CEDFCEBDAC; FEDCBAFEDC. With respect to the visual cues, the first light to flash was the hole associated with that response. For P3 and P4, the visual stimuli then became more varied in the last second of the cue, using sequences of multiple lights that change in sync with the tones. Lights could be illuminated together (as indicated by numbers in brackets) or independently. The following numbers correspond to the aperture, numbered from left to right of the operant box. The patterns for P3 were: 5434543454; (5 + 3)4(5 + 3)4(5 + 3)4(5 + 3)4(5 + 3)4. The patterns for P4 were: 1234543212; (2 + 4)(1 + 3 + 5)(2 + 4)(1 + 3 + 5)(2 + 4)(1 + 3 + 5)(2 + 4)(1 + 3 + 5)(2 + 4)(1 + 3 + 5); 1324354231; 3(2 + 4)(1 + 5)(2 + 4)3(2 + 4)(1 + 5)(2 + 4)3. The tone/light pattern played on each winning trial was determined randomly, but no pattern was presented on sequential trials. The tray light also flashed at a frequency of 5 Hz in conjunction with the array lights and tones. As for the uncued version, animals received 5 to 6 sessions per week and were tested until behavioural stability as determined by a non-significant effect of session or session x option in a repeated-measures ANOVA.
2.4 Osmotic pump implantation

Animals were subcutaneously implanted with a model 2ML4 osmotic pump (Alzet, DURECT Corporation, Cupertino, CA) delivering either 5 mg/kg/day of ropinirole hydrochloride (Tocris, R&D Systems, Minneapolis, USA) or 0.9% saline solution for 28 days. The osmotic pump allows for 28 days of chronic drug delivery, and its use has been validated in multiple experiments (e.g. Kapur et al, 2003; Kemmerer et al, 2003; Kippin et al, 2005; Vernon et al, 2012). Although the half-life of ropinirole is 5-6 hours in humans, it is only 0.5 hours in rats (Ramji et al, 1999). Acute doses in the range of 1-5 mg/kg in rats did not induce turning behaviours in a 6-OHDA model of PD nor affected spatial memory and motor functions (Fukuzaki et al, 2000; Mavrikaki et al, 2014). However, if administered chronically, these doses surpass the therapeutic range used in humans (Nashatizadeh et al, 2009). Therefore, for the experiments in this dissertation, we chose a dose that showed neuroprotective effects and reduction in anxiety, but had no effect on locomotion or cognition, although it improved motor deficits in rats overexpressing D_{2/3} receptors (Iida et al, 1999; Matsukawa et al, 2007; Millan et al, 2004; Rogers et al, 2000). The dose used here is also similar to the single daily dose of the prolonged released formulation of ropinirole used in human patients (Nashatizadeh et al, 2009).

The osmotic pumps were steriley filled with concentration of solution based on each rat’s weight a day prior to implantation and kept overnight in a sterile 50 ml falcon tube filled with 0.09% saline solution. Calculations for formulating the solution were performed using the Alzet guide. Animals were anesthetized with isoflurane (4% induction) and monitored continuously during surgery. Levels of the inhalant were adjusted (to about 2.5%) to maintain a surgical level of anesthesia throughout the surgery. Ketoprofen and bupivacaine (both from AVP Supplies) were administered subcutaneously as systemic and local analgesic, respectively.
Animals remained in their home cages for two days following surgery before resuming testing. During this time, water was available *ad libitum* and rats were fed 20 grams of laboratory rat chow per day. Animals’ recovery was monitored and recorded daily for at least 10 days. Procedures for osmotic pump removal in experiment 1 in Chapter 3 were similar to those followed for implantation.

### 2.5 Data Analyses

All analyses were conducted using SPSS (version 20, 22, or 24, SPSS/IBM corp, Chicago, USA). As per our previous publications, an arcsine transformation was performed prior to statistical analysis of variables expressed as a percentage in order to limit the effect of an artificially imposed ceiling. Behaviour was assessed at baseline, following all surgical procedures and drug administration, throughout the 28 days of ropinirole delivery, and following removal of the pump in experiment 1 in Chapter 3. The significance level for all effects was $p \leq 0.05$. Violation of the sphericity assumption revealed with Mauchly’s test was corrected using the Greenhouse-Geisser procedure.

#### 2.5.1 The rBT

All data were subjected to a within-subjects repeated-measure ANOVA. In the rBT, the key dependent variable was the percent choice of the uncertain lever, analysed as a within-subjects factor across bet size (3 levels, 1-3 sugar pellets). Behavioural data were analysed in weekly bins of 5 daily sessions with session (5 levels, sessions 1-5) as a within-subjects factor and drug treatment or lesion (2 levels, ropinirole vs saline; 6-OHDA vs sham; TH:cre positive vs TH:cre negative) as a between subjects factor. As per our previous report (Cocker *et al*, 2012a), individual rats were classified as wager-sensitive or wager-insensitive based on their choice of...
the uncertain lever as a function of bet size across 5 stable preoperative sessions. The choice of the uncertain option at each bet size was averaged and plotted in order to generate an equation of the form \( y = mx + c \) in which the factor \( m \) indicates the gradient of the line i.e. the degree to which choice of the uncertain option changed as a function of increasing bet size. Animals were classified as wager-sensitive if this value fell more than one standard deviation below a theoretical zero. This distinction was used as a between subjects factor (wager-sensitivity, 2 levels) in all analyses.

In Chapters 3-5, in order to determine whether a subgroup of individual rats responded to drug treatment and drove the effect observed, individual ANOVAs were performed on data from each rat, in which the final 5 sessions of ropinirole treatment were compared to five baseline sessions with a single within-subjects factor (time point, 2 levels). A Chi-Squared analysis on the number of responders/non-responders and wager sensitive/non-sensitive rats was then performed.

In the rBT, the following variables for non-choice behaviour were also analysed: lever choice latency; reward collection latency; hole omissions; choice omission. For lever choice latency and reward collection latency, the lever chosen (2 levels, safe vs uncertain) was also included.

### 2.5.2 The rGT and cued rGT

Similar to analyses in the rBT, data were subjected to a within-subjects repeated-measure ANOVA. Analyses in the cued rGT were identical to those performed in the standard version of the task. In these tasks, the key dependent variable was the percent choice of the various options, analysed as a within-subjects factor across options (4 levels, P1-P4). Once again, behavioural data were analysed in weekly bins of 5 daily sessions with session (5 levels, sessions 1-5) as a
within-subjects factor and drug (2 levels, ropinirole vs saline) as a between subjects factor. 
Research using the human IGT often operationalizes subjects’ performance as good or bad on the 
task by analyzing the degree to which subjects preferred the advantageous options over the 
disadvantageous options, typically subtracting the number of choices from the disadvantageous 
decks from those made from the advantageous decks. Therefore, and as per previous report 
(Barrus et al, 2015; Zeeb et al, 2009), individual animals were classified as risky or conservative 
based on their baseline preference for the advantageous or disadvantageous options across 5 
stable pre-operative sessions. The average for 5 sessions of the equation of the form \[((P1 + P2) – 
(P3 + P4))\] was calculated for each rat to determine a single score, with scores above zero 
indicating a more optimal choice strategy whereas those below zero indicated a more risk-
preferring strategy. This difference between the risky versus conservative choice strategy was 
used as a between subject factor (choice strategy, 2 levels) for all analysis. As per analysis in the 
rBT, individual response to the drug was determined using 5 sessions for each rat with time point 
and option as within-subject factors (time point: 2 levels, pre-drug treatment vs drug treatment; 
option: 4 levels, P1-4).

In the rGT, the following variables for non-choice behaviour were analysed: percent 
premature responses; choice latency; collection latency; omissions; trials completed. The 
percentage of trials on which an animal chose a particular option was calculated according to the 
following formula: number of choices of a particular option / number of total choices made x 
100. The percentage of choices, rather than a raw count of responses, was used to determine 
preferences and analysed in an ANOVA as a within-subject factor (4 levels, P1-4). This ensured 
that either individual variation or drug induced changes in the number of trials completed (which 
could itself be influenced by changes in response latencies or premature responding) would not
be interpreted as genuine differences in choice preference; animals had to choose an option proportionally more or less relative to the other options, regardless of the absolute number of responses made. For the other parameters that were not separated by choice such as premature responses, trials, omissions, collection latency and choice latency, session was the only within-subjects factor. As with analysis of data from the 5CSRT, the percent of premature responses made was calculated as the number of premature responses made / total number of trials initiated x 100.
Chapter 3: Effect of chronic ropinirole on the rBT in healthy rats and in a rat model of PD Introduction

Chronic D2/3 agonist ropinirole treatment increases preference for uncertainty in rats regardless of baseline choice patterns

3.1 Introduction

Recently developed dopamine D2/3 receptor agonist drugs are highly effective at ameliorating motor and neuropsychiatric symptoms of PD. However, they can engender ICDs such as GD in a subgroup of patients. These DRT-induced ICDs result in significant psychosocial distress and serious impairments in quality of life that can surpass the disability caused by the disease in patients prescribed these drug therapies (Voon et al, 2011b; Weintraub et al, 2010; Weintraub et al, 2006a; Weintraub et al, 2006b). It has also been shown that DRT can precipitate ICDs in other patient groups for which they are prescribed, including restless leg syndrome (Voon et al, 2011c).

Due to the possible devastating side-effects DRTs may engender in a minority of patients, neurologists may hesitate to prescribe these drugs although their effectiveness and lower propensity for developing dyskinesia has been established (i.e. Zhang and Tan, 2016). Some studies suggest that both a previous history of ICDs in the patient or their families may confer vulnerability (Voon et al, 2007), although this does not account for every case (Voon et al, 2011c).

1 A version of this chapter has been previously published in the manuscript by Tremblay M, Silveira MM, Kaur S, Hosking JG, Adams WK, Baunez C and Winstanley CA. (2016). “Chronic D2/3 agonist ropinirole treatment increases preference for uncertainty in rats regardless of baseline choice patterns”. European Journal of Neuroscience 45(1): 159-166. doi: 10.1111/ejn.13332
2011d). It is therefore unclear if the change in risky choice in PD patients may be attributed to a combination of premorbid factors and DRT, or to individual differences in the physiological reaction to DRT that may depend on, or be independent from, PD.

Similar to individuals with GD, PD patients show deficits in a variety of decision-making tasks. Individuals with problem gambling make higher rates of risky choices even when the probability of reward decreases on a probability discounting task (Madden et al, 2009), and PD patients on DRTs (including dopamine agonists and levodopa) show increased risky decisions on probabilistic tasks such as the Game of Dice (Brandt et al, 2015; Labudda et al, 2010). Recent work suggests that repeated and chronic administration of pramipexole, a dopamine D2/3 agonist used in the treatment of PD, increases choice of the risky option on a probability discounting task in both healthy rats and in a rat model of PD (Holtz et al, 2016; Rokosik et al, 2012). These landmark findings therefore suggest that DRT alone is primarily responsible for the behavioural changes observed.

The current study using an animal model of decision making was designed to further test this important hypothesis, and also to determine whether individual differences in basal preference for uncertain outcomes influenced response to the drug. We therefore assessed whether chronic administration of the dopamine D2/3 agonist ropinirole altered performance of the rodent Betting task (rBT; Cocker et al, 2012a). This task, which simply measures preference for certain vs. uncertain rewarding outcomes of equal utility, reliably allows for the objective observation of marked individual differences in choice; as the amount of reward at stake increases, some rats shift their preference towards the guaranteed outcome, whereas the choice of others remains indifferent to the change in wager size (Cocker et al. 2012b, Tremblay et al 2014). Ropinirole was delivered via osmotic mini-pump in order to assess whether behavioural
change would still be evident using a “slow-release” formulation similar to that used with pramipexole in a previous study (Holtz et al., 2016), as opposed to pulsatile drug delivery (Rokosik and Napier 2012). We subsequently independently replicated the behavioural effects of chronic ropinirole in rats with selective lesions of the dopaminergic fibres innervating the dorsolateral striatum, reminiscent of early-stage PD (Baunez et al., 2007; Baunez et al., 1995; Holtz et al., 2016; Rokosik et al., 2012).

3.2 Additional Methods

3.2.1 Experiment 1: Chronic ropinirole on the rBT in healthy rats

Subjects. Subjects (n = 24) were trained to perform the rBT as described previously (Cocker et al., 2012a; Tremblay et al., 2014). Briefly, subjects chose between a safe lever that delivered a guaranteed amount of sugar reward, or an uncertain lever which delivered either double the safe reward or nothing with 50:50 odds (see Figure 2.2). The bet size in play was equivalent to the number of response holes illuminated at the start of each trial. Training continued until a stable pattern of choice was established (total sessions to behavioural stability: 64).

Osmotic pump implantation. Animals (n = 24) were divided into two groups of rats, matched for their baseline performance. Rats were anesthetized with isoflurane and subcutaneously implanted with an osmotic pump delivering either 5 mg/kg/day of ropinirole hydrochloride (n = 12) or 0.09% saline solution (n = 12) for 28 days. The pump was then removed and behavioural training continued for a further 4 weeks. Techniques for removal of the pumps were similar to those for implantation.
3.2.2 Experiment 2: Chronic ropinirole on the rBT in 6-OHDA model of early PD

Subjects. Similar to experiment 1, subjects (n = 44) were trained to perform the rBT (see Figure 2.2). Training continued until stability in behaviour was achieved (total sessions: 46). Animals in this experiment were then trained intermittently for a further 5 months prior to receiving bilateral dorsolateral striatal 6-OHDA lesions to approximate the mid-life age at which early-onset PD occurs.

Bilateral dorsolateral striatal 6-OHDA lesion surgery. The bilateral dorsolateral striatal 6-OHDA lesion rat model is commonly used as a model for early PD and features loss of dopamine terminals similar to that observed in PD (Baunez et al., 2007; Blesa et al., 2012; Deumens et al., 2002; Przedborski et al., 1995). In PD patients, dopamine neurons of the substantia nigra pars compacta begin to die. Since bilateral lesion to this area in rodents leads to severe motivational deficits that could interfere with our task performance (see Magnard et al., 2016 for review), the bilateral dorsolateral striatal 6-OHDA lesion model was used instead as a proxy for early-stage PD. Although lesioned animals may show persistent forelimb motor impairment following striatal lesions, this model allows performance of complex behavioural tasks without development of side biases (Baunez et al., 2007; Blesa et al., 2012; Deumens et al., 2002; Holtz et al., 2016; Przedborski et al., 1995; Rokosik et al., 2012). Here, animals (n = 43) were first trained on the rBT and divided into two groups matched for baseline performance. Rats then received either bilateral dorsolateral striatal lesions (n = 22) containing 12 µg/3 µl of 6-OHDA, or sham-lesion (n = 21) using standard stereotaxic techniques. Coordinates were: anteroposterior (AP): +0.2 mm; mediolateral (ML): ±3.5 mm; dorsoventral (DV): -4.5, taken from bregma, midline, and skull, respectively; incisor bar at -3.3. Following recovery, animals
were then trained on the rBT for two weeks in order to assess the effect of lesion on performance.

**Osmotic pump implantation.** After two weeks of behavioural training on the rBT following 6-OHDA lesion surgery, and once behavioural stability had been reached, animals were again divided into two matched groups, and implanted with a pump delivering either ropinirole (n = 22; 11 lesioned rats, 11 sham rats) or saline (n = 21; 11 lesioned rats, 10 sham rats) for 28 days as per experiment 1. In this experiment, tyrosine hydroxylase immunohistochemistry was performed to determine the extent of 6-OHDA lesions.

**Tyrosine Hydroxylase Immunostaining.** All animals were humanely euthanized by live decapitation. In experiment 2, tyrosine hydroxylase immunohistochemistry was performed to determine the extent of 6-OHDA lesions. Coronal sections from the striatum were sliced at 20 µm on a cryostat and mounted on glass slides coated with 2% cryo-gel and kept at -80ºC. Sections were stained on slides for tyrosine hydroxylase using a standard avidin biotin peroxidase complex (ABC) technique (Vector laboratories, Vectastain ABC kit). Tissue was fixed on slide by submersion in pre-cooled acetone (-20 degrees) for 10 minutes. Agents were pipetted carefully onto the slides and rinsed with phosphate-buffered saline (PBS). Endogenous peroxidase was destroyed using a solution of 3% hydrogen peroxide for 15 min. Slides were then incubated with blocking buffer solution of PBS containing 0.3% Triton X and 3% normal goat serum (NGS) for 1h at room temperature after which the slides were incubated for 48 h at 4 degrees with primary antibody containing 10% Triton X, 1% NGS and the primary antibody, a purified rabbit polyclonal in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg.ml BSA and 50% glycerol. Slides were then incubated with secondary antibody containing a dilution of 1:200 biotinylated goat anti rabbit (Vector laboratories, Vectastain ABC kit) for 1.5 h after which, they
were incubated with an ABC reagent preparation in PBS for another 1.5 h (Vectastain ABC kit). A reaction with DAB substrate containing the DAB stock solution and H$_2$O$_2$ (according to the DAB substrate kit for peroxidase; Vector laboratories) was then run on ice for 2-10 min. Once the reaction was stopped by several washings with PBS, the slides were coverslipped and the extents of the lesions were determined and mapped with reference to a neuroanatomical rat brain atlas (Paxinos and Watson, 1998). See Figure 3.2 A.

### 3.2.3 Data analysis

Analyses were conducted using SPSS (version 22, SPSS/IBM, Chicago, USA). Behaviour was assessed at baseline prior to all surgical procedures, following 6-OHDA lesions, and throughout the 28 days of ropinirole delivery. In experiment 1, behaviour was also assessed for four weeks following removal of the osmotic pump. Data were analysed in weekly bins of 5 daily sessions. Data were subjected to a within-subjects repeated-measure ANOVA with session (5 levels, sessions 1-5) as a within-subjects factor and drug (2 levels, ropinirole vs saline) as a between-subjects factor. The Greenhouse-Geisser correction was applied whenever the assumption of sphericity was violated. In the rBT, the key dependent variable was the percent choice of the uncertain lever, analysed as a within-subjects factor across bet size (3 levels, 1-3 sugar pellets). As per our previous report (Cocker et al, 2012a), individual rats were classified as wager-sensitive or wager-insensitive based on their choice of the uncertain option as a function of bet size, and this between subjects factor was also included in all analyses (wager-sensitivity, 2 levels). In the model of early PD, surgery group (2 levels, sham vs lesion) was also added as a between-subjects factor. In order to determine if this response to ropinirole was present in all rats, or just within a subgroup of responders, individual ANOVAs were performed on data from each rat, in which the final 5 sessions of ropinirole treatment were compared to five baseline
sessions with a single within-subjects factor (time point, 2 levels). A Chi-Squared analysis on
the number of responders/non-responders and wager sensitive/non-sensitive rats was then
performed.

3.3 Results

3.3.1 Experiment 1: Chronic ropinirole on the rBT in healthy rats

Choice behaviour. Wager-insensitive rats (n = 14) sampled fairly equally from both the
safe and uncertain levers, regardless of the bet size in play (bet size: $F_{2,26} = 0.02, p = 0.982, \text{NS}$),
whereas wager-sensitive animals (n = 10) drastically decreased their preference for the uncertain
reward as the bet size increased (bet size: $F_{2,18} = 53.74, p < 0.001$). Chronic ropinirole increased
choice of the uncertain lever across all bet sizes, regardless of animals’ baseline preference for
the safe or uncertain option (Figure 3.1 A-B; week 4- treatment group: $F_{1,20} = 10.89, p = 0.004$;
treatment group x bet size; Figure 3.1 C-F; treatment group x wager-sensitivity: all Fs < 2.46, all
$p_s > 0.098, \text{NS}$). This change was evident from the second week following osmotic pump
implantation and remained significant for the remaining of the 28 days of drug administration
(Table 3.1; drug treatment group- week 1: $F_{1,20} = 1.15, p = 0.296, \text{NS}$; week 2: $F_{1,20} = 5.52, p =
0.029$; week 3: $F_{1,20} = 9.70, p = 0.005$; drug treatment group x wager-sensitivity: all Fs < 0.54, all
$p_s > 0.471, \text{NS}$; drug treatment x bet size x sensitivity: $F_{2,40} = 1.54, p = 0.226, \text{NS}$; ropinirole
only: bet size x sensitivity x response: $F_{2,16} = 0.18, p = 0.730, \text{NS}$).
Figure 3.1 Choice behaviour on the rBT during chronic ropinirole in healthy rats

Figure 3.1. Chronic ropinirole increased choice of the uncertain lever on the rBT, which did not depend on baseline preference for the safe or uncertain option. Data is shown pre-drug treatment, during drug treatment, and after withdrawal. Percent choice of the uncertain lever in the saline (A) and in the ropinirole treated animals (B). As a group, rats treated with ropinirole increased their choice of the uncertain lever, a behaviour which returned to baseline following withdrawal. Percent choice of the uncertain lever in the wager-sensitive (C-D) and wager-insensitive rats (E-F) treated with saline or ropinirole. Choice behaviour in the non-responder (G) and responder rats (H). Data shown are mean ± SEM. Used with permission from Tremblay and colleagues (Tremblay et al, 2016).
Table 3.1  Choice behaviour on the rBT during chronic ropinirole in healthy rats

<table>
<thead>
<tr>
<th>Time point</th>
<th>Bet Size</th>
<th>Pre-drug treatment</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wager-Insensitive</td>
<td>1</td>
<td>42.68 ± 8.23</td>
<td>39.95 ± 9.23</td>
<td>41.49 ± 8.32</td>
<td>39.81 ± 8.15</td>
<td>37.35 ± 5.77</td>
<td>40.50 ± 6.52</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
<td>2</td>
<td>47.04 ± 10.87</td>
<td>45.28 ± 10.63</td>
<td>43.82 ± 9.65</td>
<td>41.65 ± 9.92</td>
<td>40.93 ± 8.97</td>
<td>43.47 ± 8.04</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
<td>3</td>
<td>43.96 ± 11.83</td>
<td>44.01 ± 12.69</td>
<td>37.55 ± 9.73</td>
<td>37.10 ± 9.67</td>
<td>38.07 ± 9.67</td>
<td>35.04 ± 9.60</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
<td>1</td>
<td>59.89 ± 13.65</td>
<td>61.97 ± 11.83</td>
<td>59.83 ± 12.69</td>
<td>57.46 ± 10.59</td>
<td>55.89 ± 10.21</td>
<td>64.66 ± 6.15</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
<td>2</td>
<td>44.82 ± 8.16</td>
<td>44.14 ± 6.89</td>
<td>42.55 ± 5.89</td>
<td>40.77 ± 5.27</td>
<td>42.03 ± 5.76</td>
<td>45.33 ± 6.15</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
<td>3</td>
<td>23.59 ± 19.83</td>
<td>16.33 ± 5.89</td>
<td>15.09 ± 6.27</td>
<td>16.41 ± 4.59</td>
<td>22.61 ± 6.54</td>
<td>22.61 ± 6.54</td>
</tr>
<tr>
<td>Ropinirole</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
<td>1</td>
<td>48.57 ± 7.61</td>
<td>54.43 ± 6.29</td>
<td>61.28 ± 5.53</td>
<td>65.52 ± 5.22</td>
<td>64.52 ± 4.46</td>
<td>51.38 ± 6.18</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
<td>2</td>
<td>48.32 ± 10.21</td>
<td>58.12 ± 8.50</td>
<td>61.08 ± 10.32</td>
<td>60.52 ± 10.09</td>
<td>63.47 ± 10.22</td>
<td>55.11 ± 9.29</td>
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<tr>
<td>Wager-Sensitive</td>
<td>3</td>
<td>49.12 ± 10.91</td>
<td>55.93 ± 10.95</td>
<td>61.11 ± 10.77</td>
<td>61.63 ± 11.28</td>
<td>65.41 ± 12.09</td>
<td>55.68 ± 9.59</td>
</tr>
<tr>
<td>Ropinirole</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
<td>1</td>
<td>66.09 ± 3.38</td>
<td>62.83 ± 2.84</td>
<td>69.79 ± 4.38</td>
<td>74.51 ± 5.76</td>
<td>77.99 ± 6.16</td>
<td>51.82 ± 5.51</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
<td>2</td>
<td>39.87 ± 4.94</td>
<td>50.12 ± 2.44</td>
<td>61.74 ± 6.35</td>
<td>73.14 ± 7.46</td>
<td>73.12 ± 8.52</td>
<td>38.32 ± 4.67</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
<td>3</td>
<td>15.94 ± 5.45</td>
<td>33.93 ± 3.47</td>
<td>56.54 ± 8.78</td>
<td>66.64 ± 9.89</td>
<td>66.93 ± 10.28</td>
<td>27.88 ± 6.44</td>
</tr>
</tbody>
</table>
The ropinirole-induced increase in choice of the uncertain option was evident not only when the saline group was compared to the ropinirole group, but also when each drug treatment group was compared to its respective baseline prior to surgery (Figure 3.1 A-B; drug treatment week 4 vs baseline: drug treatment group x time point: $F_{1,20} = 29.65, p < 0.001$; ropinirole-treated: $F_{1,10} = 26.52, p < 0.001$; saline-treated: $F_{1,10} = 3.84, p = 0.078$, NS). Four weeks after the osmotic pump was removed, choice behaviour in the ropinirole- and saline-treated groups was no longer significantly different (withdrawal week 4, treatment group: $F_{1,20} = 0.44, p = 0.515$, NS; treatment group x wager-sensitivity: $F_{1,20} = 1.08, p = 0.310$, NS).

Human patients differ in sensitivity to dopamine agonist therapy. Contrary to our expectations, the degree of wager-sensitivity did not affect the response to ropinirole, as would be expected if predisposition to gambling behaviour was responsible for the development of ICDs. We performed additional analyses to determine whether a subgroup of rats drove the effect of ropinirole, or if instead this increase in uncertain choices was universal. These analyses identified 8 out of 12 rats as “responders” - those who increased their choice of the uncertain lever in response to ropinirole- split equally between the wager-sensitive (n = 4) and wager-insensitive (n = 4) subgroups (Figure 3.1 G-H; drug treatment week 4 vs baseline, responders vs non-responders: time point x group: $F_{1,8} = 13.35, p = 0.006$; responders only, time point: $F_{1,6} = 52.10, p < 0.001$; non-responders only; time point: $F_{1,2} = 2.59, p = 0.249$, NS; analysis by wager-sensitivity; all Fs < 4.10, all ps > 0.089, NS; number of responders/non-responders and wager-sensitive/wager-insensitive: $\chi^2(1) = 0.686, p = 0.408$, NS).

Non-choice variables. Data values for all non-choice variables measured in the rBT are given in Table 3.2. Somewhat tracking changes in choice preference, rats performed more trials while receiving ropinirole, regardless of baseline preference for the safe or uncertain option,
from week 2 onwards (drug treatment group- drug treatment week 1: $F_{1,20} = 0.57, p = 0.458, \text{NS}$; week 2: $F_{1,20} = 5.45, p = 0.030$; week 3: $F_{1,20} = 8.20, p = 0.010$; week 4: $F_{1,20} = 10.16, p = 0.005$; drug treatment x wager-sensitivity: all Fs < 1.38, all ps > 0.254, NS) and also made fewer hole omissions (drug treatment group-, drug treatment week 1: $F_{1,20} = 0.47, p = 0.499, \text{NS}$; week 2: $F_{1,20} = 5.56, p = 0.029$; week 3: $F_{1,20} = 8.58, p = 0.008$; week 4: $F_{1,20} = 15.21, p = 0.001$; drug treatment x wager-sensitivity: all Fs < 0.41, all ps > 0.529, NS). Lastly, ropinirole appeared to transiently reduce the latency to choose the uncertain lever during week 2 of drug administration (drug treatment week 2- drug treatment group: $F_{1,16} = 4.64, p = 0.047$; all other Fs < 3.68, all other ps > 0.073, NS; drug treatment x wager-sensitivity: all Fs < 3.92, all ps > 0.065, NS). This transient difference was small in magnitude and unlikely to be of functional significance. None of these effects were apparent following removal of the mini-pump (all Fs < 3.41, all ps > 0.763, NS). Additional analysis suggested that ropinirole affected these non-choice variables to a comparable degree in animals designated as responders and non-responders (all Fs < 4.27, all ps > 0.055, NS), with the exception that responders made slightly more hole omissions in week 2 ($F_{1,8} = 6.86, p = 0.031$, bet size x responder group: $F_{2,20} = 3.10, p = 0.067, \text{NS}$). Chronic ropinirole administration did not significantly affect the latency to collect reward or the number of choice omissions made at any time point tested (drug treatment group: all Fs < 4.43, all ps > 0.055, NS; drug treatment x wager-sensitivity: all Fs < 2.97, all ps > 0.108, NS).
Table 3.2  Other measurements during ropinirole in healthy rats

<table>
<thead>
<tr>
<th>Bet Size</th>
<th>Choice Latency-Safe</th>
<th>Choice Latency-Uncertain</th>
<th>Collection Latency-Safe</th>
<th>Collection Latency-Uncertain</th>
<th>Choice Omission</th>
<th>Hole Omission</th>
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</thead>
<tbody>
<tr>
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<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>1.81 ± 0.09</td>
<td>1.10 ± 0.10</td>
<td>0.43 ± 0.03</td>
<td>0.44 ± 0.04</td>
<td>0.03 ± 0.03</td>
<td>3.94 ± 0.95</td>
</tr>
<tr>
<td>2</td>
<td>1.83 ± 0.08</td>
<td>1.97 ± 0.07</td>
<td>0.40 ± 0.02</td>
<td>0.43 ± 0.03</td>
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<td>1.74 ± 0.58</td>
</tr>
<tr>
<td>3</td>
<td>1.88 ± 0.09</td>
<td>1.80 ± 0.03</td>
<td>0.38 ± 0.02</td>
<td>0.42 ± 0.04</td>
<td>0.00 ± 0.00</td>
<td>3.6 ± 0.75</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
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<tr>
<td>1</td>
<td>1.67 ± 0.19</td>
<td>1.51 ± 0.18</td>
<td>0.34 ± 0.02</td>
<td>0.41 ± 0.04</td>
<td>0.00 ± 0.00</td>
<td>5.08 ± 1.32</td>
</tr>
<tr>
<td>2</td>
<td>1.64 ± 0.21</td>
<td>1.60 ± 0.20</td>
<td>0.34 ± 0.02</td>
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<td>2.72 ± 0.34</td>
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<tr>
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<td>1.53 ± 0.16</td>
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<td>3.6 ± 0.71</td>
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</tr>
<tr>
<td>1</td>
<td>1.81 ± 0.07</td>
<td>1.42 ± 0.08</td>
<td>0.44 ± 0.04</td>
<td>0.38 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>1.23 ± 0.60</td>
</tr>
<tr>
<td>2</td>
<td>1.92 ± 0.14</td>
<td>1.50 ± 0.05</td>
<td>0.41 ± 0.03</td>
<td>0.38 ± 0.03</td>
<td>0.03 ± 0.03</td>
<td>0.51 ± 0.23</td>
</tr>
<tr>
<td>3</td>
<td>1.86 ± 0.14</td>
<td>1.52 ± 0.07</td>
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<td>0.06 ± 0.06</td>
<td>2.49 ± 0.53</td>
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<tr>
<td>Ropinirole</td>
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</tr>
<tr>
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<td>1.40 ± 0.09</td>
<td>1.51 ± 0.09</td>
<td>0.39 ± 0.06</td>
<td>0.39 ± 0.02</td>
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<td>1.96 ± 1.20</td>
</tr>
<tr>
<td>2</td>
<td>1.52 ± 0.09</td>
<td>1.52 ± 0.05</td>
<td>0.33 ± 0.01</td>
<td>0.38 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>0.36 ± 0.12</td>
</tr>
<tr>
<td>3</td>
<td>1.45 ± 0.05</td>
<td>1.59 ± 0.08</td>
<td>0.35 ± 0.04</td>
<td>0.37 ± 0.02</td>
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<td>2.04 ± 0.43</td>
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<tr>
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<td>3</td>
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</tr>
</tbody>
</table>
3.3.2 Experiment 2: Chronic ropinirole on the rBT in 6-OHDA model of early PD

*Effect of 6-OHDA lesion.* In this group of rats, 24 rat were designated as wager-sensitive rats due to their drastic decrease in preference for the uncertain reward as the bet size increased (bet size: $F_{4,46} = 85.24, p < 0.001$), whereas 19 were designated as wager-insensitive animals and sampled fairly equally from both the safe and uncertain levers, regardless of the bet size in play (bet size: $F_{2,36} = 3.22, p < 0.064$, NS). As is typical for this model (Baunez *et al.*, 2007; Holtz *et al.*, 2016; Riddle *et al.*, 2012; Rokosik *et al.*, 2012), 6-OHDA lesion resulted in visible loss of tyrosine hydroxylase immunostaining within the dorsolateral region of the striatum (Figure 3.2 A). However, lesion to this area did not affect choice behaviour (surgery group, surgery group x wager-sensitivity, surgery x bet size: all $F$s < 1.58, all $p$s > 0.216, NS).
Figure 3.2 6-OHDA Lesions. Choice behaviour on the rBT in the 6-OHDA model of PD

(A) Pictomicrograph representative of 6-hydroxydopamine lesion to the dorsolateral striatum. Chronic ropinirole increased choice of the uncertain lever regardless of baseline preference. Data are shown in the sham and lesioned rats pre-drug treatment and during drug treatment. Percent choice of the uncertain lever in the saline (B) and in the ropinirole treated animals (C). Similar to experiment 1, as a group, rats treated with ropinirole increased their choice of the uncertain lever when the bet size in play was larger. Choice behaviour in the non-responder (D) and responder rats (E). Data shown are mean ± SEM. Used with permission from Tremblay and colleagues (Tremblay et al., 2016).
Choice behaviour. Chronic ropinirole increased choice of the uncertain lever at the largest bet size regardless of whether the animal underwent lesion or sham surgery, or the animals’ wager-sensitivity, or (Figure 3.2 B-C; week 4- treatment group: $F_{1,35} = 3.22, p = 0.081$, NS; treatment group x bet size: $F_{2,49.47} = 5.59, p = 0.013$; treatment group x surgery group: $F_{1,35} = 0.276, p = 0.603$, NS; treatment group x wager-sensitivity: $F_{1,35} = 0.022, p = 0.883$, NS). This change only appeared during the last week of ropinirole administration (Table 3.3; drug treatment group- week 1: $F_{1,35} = 0.001, p = 0.975$, NS; week 2: $F_{1,35} = 0.87, p = 0.357$, NS; week 3: $F_{1,35} = 2.31, p = 0.138$, NS; drug treatment group x surgery group: all Fs < 0.54, all ps > 0.467, NS; drug treatment group x wager-sensitivity: all Fs < 0.05, all ps > 0.824, NS; drug treatment x bet size x sensitivity: $F_{2,78} = 0.17, p = 0.847$, NS; ropinirole only: bet size x sensitivity x response: $F_{2,36} = 2.56, p = 0.091$, NS). As per experiment 1, this effect was evident not only when the saline group was compared to the ropinirole group, but also when each drug treatment group was compared to its respective baseline prior to osmotic pump implantation (drug treatment week 4 vs baseline: drug treatment group x time point: $F_{1,35} = 28.52, p < 0.001$; ropinirole-treated: time point: $F_{1,18} = 44.17, p < 0.001$; time point x bet size: $F_{1.53,27.60} = 11.97, p < 0.001$; saline-treated: time point: $F_{1,17} = 1.06, p = 0.318$, NS; time point x bet size: $F_{1.52,25.83} = 0.53, p = 0.595$, NS).
Table 3.3 Choice behaviour on the rBT during ropinirole in the 6-OHDA model of PD

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<th>Bet Size</th>
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<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
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<tr>
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<td>50.55 ± 8.54</td>
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<td>48.90 ±</td>
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<td>51.67 ±</td>
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<td>58.26 ± 11.05</td>
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<td>69.50 ±</td>
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<td><strong>Wager-Sensitive</strong></td>
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<td>53.33 ±</td>
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<td>42.90 ± 5.51</td>
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<td>49.03 ±</td>
<td>53.43 ±</td>
<td>57.22 ±</td>
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<td>24.50 ± 7.05</td>
<td>31.80 ±</td>
<td>37.45 ±</td>
<td>40.93 ±</td>
<td>44.42 ±</td>
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</table>
Similar to experiment 1, we explored whether a subgroup of animals drove the effect of the drug. Analyses identified a similar proportion of rats (16 out of 22) as “responders”, split equally between the wager-sensitive (n = 8) and wager-insensitive (n = 8) subgroups (Figure 3.2 D-E; drug treatment week 4 vs baseline, responders vs non-responders: time point x group: $F_{1,18} = 44.17, p < 0.001$; responders only; time point: $F_{1,12} = 33.99, p < 0.001$; non-responders only; time point: $F_{1,2} = 15.01, p = 0.061$, NS; analysis by surgery group and by wager sensitivity, all Fs < 0.64, all $p$s > 0.441, NS; number of responders/non-responders and wager-sensitive/wager-insensitive: $\chi^2(1) = 0.489, p = 0.484$, NS).

*Non-choice variables.* Data values for all non-choice variables measured in the rBT in the 6-OHDA-ropinirole experiment are given in Table 3.4. Chronic ropinirole administration did not significantly affect the latency to choose an option or collect reward or the number of choice omissions made (week 4: drug treatment group: all Fs < 3.49, all $p$s > 0.070, NS; drug treatment x surgery group; all Fs < 1.81, all $p$s > 0.187, NS; drug treatment x wager-sensitivity: all Fs < 2.89, all $p$s > 0.098, NS). The drug appeared to reduce the number of response hole omitted when bet size 2 and 3 were in play during week 3 of drug administration, regardless of animal’s preference for the safe or uncertain option, or surgery group (week 3: drug treatment group: $F_{1,35} = 5.75, p = 0.022$; drug treatment x surgery group; $F_{1,35} = 0.34, p = 0.564$, NS; drug treatment x wager-sensitivity: $F_{1,35} = 0.01, p = 0.921$, NS; ropinirole x bet size: $F_{2,70} = 3.62, p = 0.032$; bet size 1: $F_{1,41} = 4.00, p = 0.052$, NS; bet size 2: $F_{1,41} = 7.49, p = 0.009$; bet size 3: $F_{1,41} = 5.34, p = 0.026$). However, contrary to the previous group of rats, this difference was temporary and only observable during that week. Also, additional analysis suggested that ropinirole affected the number of hole omission made to a comparable degree regardless of whether animals were designated as responders and non-responders (responder group: $F_{1,20} = 0.02, p = 0.889$, NS; bet
size x responder group: $F_{2,40} = 0.10, p = 0.905, \text{NS}$). Chronic ropinirole did not affect any other parameter at any other time point tested in this group of rats (choice and collection latency, and choice omission; week 1-3: drug treatment: all Fs < 2.53, all $p$s > 0.138, NS; drug treatment x surgery group: all Fs < 1.53 all $p$s > 0.240, NS; drug treatment x wager-sensitivity: all Fs < 2.53, all $p$s > 0.138, NS).
Table 3.4 Other measurements on the rBT during ropinirole in the 6-OHDA model of PD

<table>
<thead>
<tr>
<th>Bet Size</th>
<th>Choice Latency-Safe</th>
<th>Choice Latency-Uncertain</th>
<th>Collection Latency-Safe</th>
<th>Collection Latency-Uncertain</th>
<th>Choice Omission</th>
<th>Hole Omission</th>
</tr>
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<tbody>
<tr>
<td>Sham-Saline</td>
<td>1.29 ± 0.13</td>
<td>1.31 ± 0.12</td>
<td>0.39 ± 0.05</td>
<td>0.31 ± 0.04</td>
<td>0.00 ± 0.04</td>
<td>0.30 ± 0.24</td>
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<tr>
<td>2</td>
<td>1.42 ± 0.13</td>
<td>1.30 ± 0.10</td>
<td>0.37 ± 0.04</td>
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<td>0.05 ± 0.04</td>
<td>0.10 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>1.39 ± 0.12</td>
<td>1.30 ± 0.12</td>
<td>0.40 ± 0.07</td>
<td>0.30 ± 0.04</td>
<td>0.00 ± 0.04</td>
<td>0.65 ± 0.29</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
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<td>0.38 ± 0.02</td>
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3.4 Discussion

Here, we show that chronic ropinirole treatment increased preference for the uncertain option on the rBT in roughly two-thirds of subjects. The ability of ropinirole to bias choice towards the probabilistic delivery of reward on this task was not altered by 6-OHDA lesions of the dorsolateral striatum, an animal model of early PD, either with respect to the magnitude of the change in choice, or the proportion of animals affected. Even though we could detect substantial variation in subjects’ baseline preference for the probabilistic option in the rBT, these individual differences did not predict the response to ropinirole treatment. Collectively, these data support the hypothesis that the altered judgment exhibited by patients with PD or restless leg syndrome following chronic DRT administration can be largely attributed to the direct actions of the drugs themselves (Rokosik et al., 2012; Weintraub, 2008), rather than resulting from an interaction between the medication and basal risk preference, or any change in dopamine function caused by the diseases for which they were originally prescribed.

Whenever attempts are made to extrapolate from rat to human behaviour, we must consider the degree to which the rodent tasks accurately capture the cognitive trait in question. The rBT was developed as one of a series of tasks aiming to capture non-normative decision-making biases evident in human decision-making under uncertainty (see Cocker et al., 2015 for discussion). Psychobiological accounts of GD posit that these cognitive distortions can contribute to the etiology and severity of GD (Clark, 2010). Furthermore, the rBT is very similar to the “gain” condition of a gambling-like task used recently to compare choice patterns of PD patients with and without ICDs, in which PD-ICD patients exhibited significantly greater preference for uncertain outcomes (Voon et al., 2011a). As in the standard trials in Voon’s 2011
task, there was no real risk in the rBT since the expected value of both the uncertain and safe option was the same. This differs from other similar tasks used in humans in which there is a real advantage in choosing one option over the other such as in the Game of Dice (e.g. Brandt et al, 2015; Labudda et al, 2010). By maintaining utility across both options, it is possible to detect subjective choice biases without the potential confounds caused by individual differences in the evaluation of differences in net gain.

Although the proportion of rats that responded to ropinirole by increasing preference for uncertainty on the rBT is considerably higher than the incidence of ICDs after DRT therapy, this may be because all the animals are repeatedly exposed to uncertain outcomes, and this behavioural process can actually sensitize the dopamine system (Singer et al, 2012; Zack et al, 2014). This shift towards selection of probabilistic over guaranteed outcomes also matches previous work in which repeated daily injections of pramipexole caused a similar effect on a probability discounting task, again irrespective of 6-OHDA depletion of the dorsolateral striatum (Rokosik et al, 2012). Hence, a similar bias towards uncertain outcomes is observed, regardless of pulsatile or slow-release delivery, or which D2/3 agonist is used. Another study using chronic pramipexole via osmotic pump similar to the one used in this study has also found that a subset of rats increased discounting of the probabilistic option in response to the drug. However, in contrast to our study in which wager-sensitivity did not predict increase in choice of uncertainty in response to ropinirole, this other study showed an increase in discounting in drug-treated animals classified as risk takers (Holtz et al, 2016). It is possible that differences in task design may account for this differential effect. For example, in the rBT, although bet sizes vary during a session, the probability of winning on the uncertain lever does not change. In comparison, the probability discounting task requires animals to update contingencies as the session advances. In
addition, although not significant in our study, rats classified as wager-sensitive, those with an irrational decision-making style, appear to show a larger increase in preference for uncertainty due to ropinirole as compared to the wager-insensitive rats. Further studies that can differentiate the biological underpinnings of individual difference in decision making could help clarify this inconsistency. It has been found that food restriction may also increase sensitivity to the locomotor effects of stimulant drugs by increasing extracellular dopamine in response to dopamine agonist drugs and sensitizing D₂ receptors (Collins et al, 2008). Although animals in the present study were food restricted, it is unlikely that the effect on choice behaviour was due to the effect of food restriction since all rats were similarly food restricted at each experimental time point.

The mechanism by which DRTs enhance preference for uncertainty has yet to be fully determined. The effects of chronic ropinirole on the rBT are qualitatively similar to the increase in risky choice induced by amphetamine, although greater in magnitude (Cocker et al, 2012a). The degree of wager-sensitivity observed on task is also negatively correlated with D₂/₃ receptor density in the dorsal, but not ventral, striatum (Cocker et al, 2012a). Put another way, the greater the preference for the uncertain option, the greater the number of striatal D₂/₃ receptors. Brief decreases in D₂ signaling within the striatum may be necessary for learning when an expected reward fails to arrive (Cohen and Frank, 2009). The constant stimulation of D₂ receptors by chronic ropinirole could therefore mask the learning signal generated by non-reward, leading to an increase in preference for uncertain outcomes.

A growing body of literature indicates that dopaminergic receptor expression differs between drug addiction, GD, and PD with ICD, despite some overlap in the symptoms and nature of the conditions (Potenza, 2014). Individuals with long-term drug addiction show
reduction in D_{2/3} receptors in all subdivisions of the striatum, and low D_{2/3} levels increase the likelihood of cocaine self-administration in rats (see Broft and Martinez, 2012 for review). In contrast, no difference in D_{2/3} receptors was observed between individuals with GD and healthy controls (Leeman and Potenza, 2013). However, high levels of D_{2/3} receptors have been observed in PD patients using positron emission topography (PET) during early stages of the disease, a time when the risk for developing ICDs in response to DRT is higher (Dagher et al, 2009; Nikolaus et al, 2009; Voon et al, 2007), yet a recent report suggests that PD-ICD patients do not exhibit greater levels of D_{2} or D_{3} receptors as compared to PD patients without ICDs (Payer et al, 2015a). The difference observed in these populations may reflect differences in medications, which change neurotransmitter levels in the brain. This may be particularly relevant to positron emission tomography (PET) studies, which measures displacement in neurotransmitter binding, and can therefore be influenced by basal dopaminergic tone. However, gambling severity and impulsivity have been associated with greater D_{3} binding in the substantia nigra of individuals with GD (Boileau et al, 2013). Ropinirole is a D_{2/3} agonist, and therefore, it is possible that D_{3} receptor expression may also be involved in the increase in uncertain choice observed in our rats. Future rodent studies combining micro-PET and ex vivo receptor analysis would be useful to determine whether changes in striatal dopamine receptor density can explain the behavioural changes observed in response to dopamine agonist treatment, and also whether altered striatal dopamine density represents a pre-existing vulnerability marker for developing ICDs following DRTs.

With regard to the signaling mechanism by which constant stimulation of D_{2/3} receptors may impact behaviour, there are two main intracellular pathways that are likely responsible. As an inhibitory G-protein coupled receptor, D_{2} receptor activation may trigger a signaling cascade
that ultimately decreases phosphorylation of DARPP at threonine 34. Alternatively, it can also activate a different signaling pathway which increases GSK3β levels, a pathway suggested to be involved in multiple neurological and psychiatric disorders marked by impulsivity such as drug addiction, schizophrenia and bipolar disorder (Beaulieu et al, 2005; Beaulieu et al, 2004). Lithium chloride and valproic acid, both of which can decrease GSK3β, were also suggested as potential treatment for GD, and valproic acid was shown in a case study to reduce symptoms of dopamine dysregulation syndrome in L-DOPA medicated PD patients, and may help in controlling symptoms of ICDs in PD patients on DRTs (Epstein et al, 2014; Hicks et al, 2011; Sriram et al, 2013; Wang et al, 2013). Other drugs that work on the opioid, serotonin, or glutamatergic systems have also been suggested as potential treatments for drug and behavioural addiction disorders, some with promising results (see Leung and Cottler, 2009; Yau and Potenza, 2015 for review). It would be of interest to discover if these drugs can improve DRT-induced ICD in PD patients. Alternatively, a recent report suggested that modification in the OPRK1, HTR2A and DDC genes may be potential predictive genetic factors in the development of ICDs in PD patients on DRT (Kraemmer et al, 2016). Predicting which PD patients are at risk for developing ICDs in response to DRT would improve DRT outcomes and increase patients’ options when deciding between therapeutic strategies.

Chronic ropinirole also increased the number of trials completed and transiently decreased choice latency of the uncertain lever in our population of healthy rats, while the number of hole omissions was temporarily decreased in our animal model of PD. Decreased omissions may suggest an increased engagement with the task as suggested by the increase in trials in the first experiment. A previous study has also observed a significant increase in trials performed following chronic ropinirole on a rodent slot machine task (Cocker et al, 2017). This
increase in trials was interpreted as an invigoration of task performance, potentially suggesting a compulsive playing style mirroring the compulsive engagement observed in gambling and other forms of addictions. The increase in trials completed, and the decrease in hole omissions, observed here were smaller in magnitude, but may likewise suggest an increase in engagement with the task during ropinirole treatment. In addition, healthy rats administered ropinirole were faster to choose the uncertain lever. Consistent with our results, an increase in the speed of decision making following dopaminergic drug treatment has also been observed in PD patients with ICDs (Voon et al, 2010). However, we did not observe faster decision making in our animal model of PD, potentially due to the mild motor effects caused by dorsolateral striatal dopamine depletion (Rokosik et al, 2012). Nevertheless, the effect of ropinirole on these ancillary task parameters are in keeping with an increase in task engagement, and further suggest mechanisms through which ropinirole and other D2/3 agonists may facilitate the development of ICDs in PD.

In our group of healthy rats undergoing withdrawal, we did not observe symptoms of DAWS such as would have been seen with changes in any of the parameters of decision-making, even in the responder rats that showed a strong effect of the drug. It is possible that DAWS were not seen in our healthy rats that had a highly functioning dopamine system, as opposed to patients with PD. However, symptoms of DAWS were also observed in a patient with restless leg syndrome, in which the dopamine system is unaffected (Shimo et al, 2015). It is therefore possible that the dose of ropinirole used in this study may differ from that used in the clinical population and explain this lack of withdrawal effect.

In sum, the data presented here suggest that enhanced preference for uncertainty following chronic administration of a D2/3 agonist may serve as a behavioural endophenotype
approximating DRT-induced ICDs. Furthermore, vulnerability to this effect appears independent of premorbid behavioural patterns. Determining the exact genetic mutation or mechanism that results in individual differences in the response to D2-receptor stimulation, thereby conferring resilience or vulnerability to DRT-induced ICDs, remains an important goal for future research that may inform therapeutic initiatives.
Chapter 4: Effect of the chronic ropinirole on the uncued and cued rGT

Adding cues to a rat gambling task mitigates choice preference and potentiates the increase in premature responding in response to chronic D\textsubscript{2/3} agonist ropinirole

4.1 Introduction

PD patients show deficits in various decision-making tasks, which appear to depend on the degree of severity in the progression of the disease, the type of drug taken, and whether patients develop ICDs. Nonetheless, evidence for the development of highly distressing ICDs like GD in a subgroup of patients following introduction of dopamine D\textsubscript{2/3} receptor agonists such as ropinirole and pramipexole is rapidly increasing (e.g. Weintraub, 2009; Weintraub and Nirenberg, 2013). Decision making is complex and the type of decision making studied may recruit specific neural circuitry. For example, it has been documented that as PD progresses to areas of the PFC, PD patients may show impairments in cognition and executive functions that are dependent on these different frontal areas (Brown and Marsden, 1990; Hirano et al, 2012). However, even in early PD when functions of the OFC and vmPFC are relatively preserved, PD patients may still present with deficits in PFC-dependent cognition, a symptom that may well be due to the introduction of DRTs (Poletti and Bonuccelli, 2012). Deficits in attention, cognitive flexibility, and speed of cognitive processing as measured by the Stroop Task and the Wisconsin Card Sorting Task (WCST) have been described in PD patients. The cognitive functions involved in performance of these tasks are believed to be regulated by the dorsolateral PFC and anterior cingulate cortex (ACC), as well as the caudate nucleus, which is involved in working memory (Frank et al, 2001). Impairment in both of these tasks can be interpreted as an incapacity to inhibit a prepotent response, and therefore a deficit in impulse control.
Interestingly, it has been suggested that dopamine treatment can improve some of these disease-related deficits, while other impairments occur as a consequence of treatment with dopamine drugs (i.e. Cools et al, 2001). For example, deficits in reversal learning, which are believed to depend on the ventral striatum projections to the amygdala and PFC, appears to be particularly sensitive to impairment by D2/3 agonist treatment (Cools et al, 2006).

As mentioned earlier, the IGT has been used clinically to assess risky decision making and gambling behaviours (Bechara et al, 1994), and has been used to assess vulnerability to GD in PD patients. In this task, participants choose between four decks of cards, each associated with various sizes of gains and penalties. Over time, participants learn to favour the two advantageous decks, which are those associated with small gains per trials but also smaller penalties, in order to accrue money. Many studies suggest that PD patients have impaired decision making on the IGT, even though their executive functions are unaffected (Gescheidt et al, 2012; Kobayakawa et al, 2008; Mimura et al, 2006; Pagonabarraga et al, 2007). However, it is difficult to disentangle the contribution of disease, drug, and ICDs in the deficits observed from clinical data alone. For example, a study showed that PD patients with GD adopted a less advantageous decision-making strategy on the IGT compared to those without GD (Rossi et al, 2010), and deficits in IGT performance were also reported in a patient with early-onset PD who developed GD following treatment with L-DOPA and then DRT (Pignatti et al, 2012).

Conversely, another study reported only trend-level increases in risky choice in a cohort of PD patients with ICDs, despite higher scores on the Barratt Impulsivity Scale (Bentivoglio et al, 2013), while another group found that PD patients without ICDs were unimpaired on the IGT (Euteneuer et al, 2009).
Deciphering the contribution of dopamine D_{2/3} agonist in creating deficits on the IGT, independent of PD or basal patterns of risky choice, could help us understand the development of GD in PD patients on DRT medication. However, establishing this relationship in the clinical population is difficult due to the high variability in human experiences. Animal studies using the rGT can therefore be of significant benefit in this regard. In Chapter 3, we demonstrated that chronic ropinirole leads to dramatic increases in preference for uncertain outcomes on the rat Betting task (rBT), which measures preference for certain vs. uncertain rewarding outcomes of equal utility (Tremblay et al., 2016). However, it is possible that decision-making under risk tested with the IGT/rGT may be controlled by different neural mechanisms (see Cocker et al., 2015 for review), in which case investigation of the effect of chronic dopamine replacement therapy on a rodent task that taps into risky decision making is necessary. The rat Gambling task (rGT) is analogous to the IGT used clinically, and measures both risky decision making and motor impulsivity (Zeeb et al., 2009). Similar to decision making on the IGT, the rGT requires rats to balance the probability of receiving smaller or larger rewards against the chances of shorter vs longer time-outs in order to maximize sugar pellet profits. As in the IGT, the optimal strategy is to avoid the tempting high-risk high-reward options, and instead favour those associated with smaller per-trial gains but also lower penalties. Impulsivity encompasses multiple constructs such as a fast decision making style with lack of consideration for future consequences or reflexion impulsivity, intolerance of delays to gratification, and reduced inhibitory control or motor impulsivity (see Evenden, 1999; Winstanley et al., 2006 for review). In the rGT, premature responses are a measure of motor impulsivity in that they measure an inability of the rats to withhold from making a response before the ITI has ended.
In addition, given the influence of cues in the pathology of drug addiction and in the transition from recreational gambling to disordered gambling (Grant et al, 2015; Obrien et al, 1992; van Holst et al, 2012a), we tested whether adding cues to the “standard” rGT would influence response to chronic ropinirole. It has been shown that cues which predict rewards increase the release of dopamine (Schultz, 1998), and our laboratory has shown that adding complex audiovisual cues associated with wins in the rGT increases the proportion of rats preferring the option with maximal uncertainty, P3, rewarded on 50% of trials (Barrus et al, 2016b). Previous studies have shown that repeated exposure to stimuli that predict uncertain outcomes can sensitize the dopamine system (Singer et al, 2012; Zack et al, 2014), suggesting that exposure to cues associated with wins on the cued rGT may likewise sensitize the dopamine system and potentially exacerbate the effect of chronic ropinirole on this task. Individual differences in cue-induced maladaptive decision-making on the cued rGT could also reveal a vulnerability to the effect of ropinirole. Therefore, here we compared the effect of chronic ropinirole on both risk-prefering and optimal decision-makers performing the cued and uncued rGT.

4.2 Additional Methods

4.2.1 Task training

Subjects were trained to perform the “standard” uncued rGT (n = 24), or the cued rGT (n = 40) as described previously and in Chapter 2 (Barrus et al, 2015; Barrus et al, 2016b; Zeeb et al, 2009). In this task, each hole is associated with a different probability of reward and punishment (Figure 2.3). The structure of the cued rGT was identical to that of the original uncued rGT except for the introduction of audiovisual cues that accompanied reward delivery on
winning trials. Comparable to the experience of human gambling games, the complexity and salience of win-associated cues increased with the win size, as shown in Table 2.1. In both tasks, the location of the pellet choice options (P1–4) was counterbalanced across animals such that half the animals were tested on version A (n = 32) and half on version B (n = 32). Animals received 5 daily sessions per week and were tested until a statistically stable pattern of choice was observed over 5 sessions (total sessions to behavioural stability: the rGT: 76; the cued rGT: 51).

4.2.2 Osmotic pump implantation

In each of the cued and uncued rGT experiment, rats were divided into two equivalent groups of animals matched for their baseline performance and subcutaneously implanted with an osmotic pump delivering either 5 mg/kg/day of ropinirole hydrochloride (see Chapter 2; rGT: n = 12; cued rGT: n = 20) or 0.09% saline solution (rGT: n = 12; cued rGT: n = 20) for 28 days. The dose of ropinirole used here was based on previous experiments, including those documented in Chapter 3 (Cocker et al, 2017; Tremblay et al, 2016). At the end of the 28 day period, rats were humanely euthanized by live decapitation.

4.2.3 Data analysis

Analyses were conducted using SPSS (version 22, SPSS/IBM, Chicago, USA). Data from baseline and from four weeks of post-operative testing were analysed in weekly bins of 5 daily sessions. Data were subjected to a within-subjects repeated-measure analysis of variance (ANOVA) with session (5 levels, sessions 1-5) as a within-subjects factor and drug (2 levels, ropinirole vs saline) as a between subjects factor. In both the cued and uncued rGT, the key dependent variable was the percent choice of each option, analysed as a within-subjects factor (option, 4 levels, P1-4). Animals were classified as risk-preferring or optimal decision-makers.
based on their net preference for the advantageous options as measured by a score variable 
([P1+P2] – [P3+P4], (see Barrus et al, 2015). This distinction was used as a between subjects 
factor (group, 2 levels). An arcsine transformation was performed prior to statistical analysis of 
variables expressed as a percentage in order to limit the effect of an artificially imposed ceiling. 
Individual response to the drug was determined using 5 sessions for each rat with timing and 
option as within-subject factors (timing: 2 levels, pre-drug treatment vs drug treatment; option: 4 
levels, P1-4). A regression analysis was also performed in order to determine if advantageous or 
disadvantageous choice behaviour on the cued/uncued rGT could predict any increase in 
premature responses following chronic ropinirole. The following variables for non-choice 
behaviour were also analysed: percent premature responses; choice latency; collection latency; 
omissions; trials completed.

4.3 Results

4.3.1 The uncued rGT

Choice behaviour. As a group, rats performing the uncued rGT adopted the best strategy 
on average, and chose the most advantageous option P2 in a larger proportion, showing option 
preference in this manner: P2 > P4 > P3 > P1. However, individual differences in preference for 
the various options at baseline led to the classification of rats as having adopted either a risk-
preferring (n = 11) or optimal (n = 13) decision-making strategy (option: $F_{3,66} = 20.74, p < 
0.001; option x choice strategy: $F_{3,66} = 20.84, p < 0.001$). Optimal decision-makers chose the 
best option (P2) more frequently than all other options, choosing options in this order: P2 > P4 > 
P3 > P1. In contrast, the risky rats showed a different pattern of choice, preferring the most 
disadvantageous option, in this manner: P4 > P3 > P2 > P1. Although the optimal pattern of
choice based on the probability of reward associated with the different option should be $P_2 > P_1 > P_3 > P_4$, rats were clearly sampling all of the different options, and some rats more than others were tempted by those with a possibility of higher reward.

Chronic ropinirole did not affect choice of the different options on the rGT at any time point, independent of baseline choice strategy (Figure 4.1 A-B; Table 4.1; drug treatment: all Fs $< 3.18$, all $p$s $> 0.090$, NS; choice x drug treatment: all Fs $< 0.18$, all $p$s $> 0.913$, NS; Figure 4.1 C-F; drug treatment x choice strategy: all Fs $< 0.96$, all $p$s $> 0.339$, NS). There was also no effect of ropinirole on decision making when comparing any particular week of ropinirole treatment to baseline behaviour (drug treatment week 1-4 vs baseline: drug treatment group x time point: all Fs $< 3.99$, all $p$s $> 0.060$, NS; ropinirole-treated: time point: all Fs $< 4.19$, all $p$s $> 0.068$, NS; time point x option: all Fs $< 0.47$, all $p$s $> 0.704$, NS; saline-treated: time point: all Fs $< 0.87$, all $p$s $> 0.374$, NS; time point x option: all Fs $< 0.83$, all $p$s $> 0.490$, NS).
Figure 4.1 Choice behaviour on the uncued rGT during chronic ropinirole

Figure 4.1. Chronic ropinirole did not alter choice strategy on the uncued rGT. Data is shown pre-drug treatment and during drug treatment. Percent choice of the various options in saline (A) and in the ropinirole treated animals (B). Percent choice of the various options in optimal decision-makers (C-D), and in risk-prefering rats (E-F). Data shown are mean ± SEM.
Table 4.1  Choice behaviour on the rGT during chronic ropinirole

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<td>P2</td>
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<td>18.40 ± 5.77</td>
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<tr>
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<td>P4</td>
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<td>10.59 ± 3.86</td>
<td>10.47 ± 3.96</td>
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<tr>
<td></td>
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<td>5.09 ± 2.37</td>
<td>2.16 ± 0.93</td>
<td>2.09 ± 0.93</td>
<td>1.57 ± 0.88</td>
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<td>16.32 ± 5.87</td>
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<td></td>
<td>P3</td>
<td>40.34 ± 14.48</td>
<td>43.46 ± 14.33</td>
<td>44.23 ± 15.86</td>
<td>42.54 ± 14.90</td>
<td>44.55 ± 15.18</td>
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<tr>
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<td>2.63 ± 0.80</td>
<td>5.64 ± 2.86</td>
<td>6.74 ± 3.38</td>
<td>5.46 ± 2.49</td>
<td>3.40 ± 1.68</td>
</tr>
<tr>
<td>Ropinirole</td>
<td>P1</td>
<td>81.96 ± 6.50</td>
<td>76.92 ± 6.77</td>
<td>79.76 ± 6.78</td>
<td>84.19 ± 4.85</td>
<td>87.09 ± 4.70</td>
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<td>P2</td>
<td>4.66 ± 2.45</td>
<td>9.16 ± 4.10</td>
<td>5.80 ± 2.67</td>
<td>6.36 ± 3.26</td>
<td>6.91 ± 4.48</td>
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<td>P3</td>
<td>10.75 ± 5.95</td>
<td>8.28 ± 4.21</td>
<td>7.71 ± 4.65</td>
<td>3.98 ± 1.70</td>
<td>2.60 ± 1.17</td>
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</table>
Similar to the analysis performed in previous experiments in Chapter 3, we investigated whether individual rats responded to the drug. Although 6 rats from the risky group could arguably form a responder group, this change in behaviour was not robust enough to significantly differentiate a responder group from the larger pool of non-responders (baseline vs drug-treatment- timing x group: \( F_{1,10} = 0.163, p = 0.695, \text{NS} \)).

Non-choice variables. Ropinirole significantly increased the number of premature responses made during the first week of administration, regardless of rats’ baseline preference for the various options, but this effect was no longer evident subsequently (Figure 4.2 A-B; drug treatment- week 1: \( F_{1,20} = 4.70, p = 0.042 \); all other weeks: Fs < 2.67, all ps > 0.118, NS; Figure 4.2 C-F; drug treatment x choice strategy: all Fs < 0.81, all ps > 0.379, NS). Choice preference for the advantageous or disadvantageous options at baseline did not predict the increase in premature responses following chronic ropinirole (Figure 4.3; ropinirole treated only: \( F_{1,10} = 0.97, p = 0.348, R^2 = 0.088, \text{NS} \)).
Figure 4.2. Chronic ropinirole increased premature responding during the first week of chronic ropinirole on the uncued rGT, regardless of baseline choice strategy. Data is shown pre-drug treatment and during drug treatment. Percent premature response made in the saline treated rats (A), and in ropinirole treated rats (B). Premature responses made in the optimal decision-makers (C-D), and in risk-preferring animals (E-F). Data shown are mean ± SEM.
Figure 4.3. Regression analysis between the score for choice on the uncued rGT and premature responding during ropinirole. Score on the uncued rGT did not predict premature responses induced by chronic ropinirole. Data shown are mean for each rat.
Data values for all other non-choice variables are given in Table 4.2. Ropinirole decreased omissions during weeks 2 and 3 of drug administration, independent of rats’ decision-making strategy, but again this effect had dissipated by week 4 (drug treatment - week 1: \( F_{1,20} = 0.04, p = 0.844, \text{NS} \); week 2: \( F_{1,20} = 6.26, p = 0.021 \); week 3: \( F_{1,20} = 7.41, p = 0.013 \); week 4: \( F_{1,20} = 2.51, p = 0.129, \text{NS} \); choice strategy: all Fs < 1.26, all ps > 0.275, NS; drug treatment x choice strategy: all Fs < 0.50, all ps > 0.488, NS). At baseline, risk-preferring rats were faster to collect reward, an effect that remained unchanged for the duration of drug treatment (choice strategy: \( F_{1,20} = 16.74, p = 0.001 \); all other Fs > 10.58, ps < 0.004). Optimal decision-makers also consistently performed more trials, likely due to the more frequent occurrence of longer punishments arising from risky rats’ preference for the disadvantageous options (baseline - choice strategy: \( F_{1,20} = 32.46, p < 0.001 \); all other Fs > 20.22, ps < 0.001). However, ropinirole treatment did not alter the number of trials performed, or latency to choose an option (drug treatment: all Fs < 3.97, all ps > 0.060, NS; drug treatment x choice strategy: all Fs < 1.47, all ps > 0.239, NS). Other than the reward collection latency and trials completed, there were no other difference between risk-preferring and optimal decision-makers (premature responses, choice latency, omission; choice strategy: all Fs < 4.25, all ps > 0.051).
Table 4.2 Other measurements on the rGT during chronic ropinirole

<table>
<thead>
<tr>
<th></th>
<th>Premature Responses</th>
<th>Choice Latency</th>
<th>Collection Latency</th>
<th>Omission</th>
<th>Trials</th>
</tr>
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<td><strong>Saline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk-preferring</td>
<td>28.15 ± 7.18</td>
<td>1.12 ± 0.21</td>
<td>0.68 ± 0.04</td>
<td>0.24 ± 0.18</td>
<td>64.48 ± 9.37</td>
</tr>
<tr>
<td>Optimal decision-</td>
<td>14.08 ± 2.38</td>
<td>0.89 ± 0.10</td>
<td>0.88 ± 0.05</td>
<td>0.49 ± 0.27</td>
<td>110.94 ± 9.06</td>
</tr>
<tr>
<td>makers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ropinirole</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk-preferring</td>
<td>26.39 ± 7.33</td>
<td>0.74 ± 0.17</td>
<td>0.69 ± 0.16</td>
<td>0.10 ± 0.10</td>
<td>60.47 ± 4.83</td>
</tr>
<tr>
<td>Optimal decision-</td>
<td>20.33 ± 5.25</td>
<td>0.75 ± 0.07</td>
<td>1.12 ± 0.08</td>
<td>0.13 ± 0.11</td>
<td>125.33 ± 10.33</td>
</tr>
<tr>
<td>makers</td>
<td></td>
<td></td>
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</table>
4.3.2 The cued rGT

Choice behaviour. Similar to previous data using this task (Barrus et al., 2016b), a high proportion of rats performing the cued rGT adopted a risky decision-making strategy (risk-preferring: n = 30; optimal decision-makers n = 10; option: $F_{3,114} = 15.64, p < 0.001$; option x choice strategy: $F_{3,114} = 21.07, p < 0.001$). Although the optimal decision-makers showed similar choice preference to those performing the standard rGT, choosing the most advantageous option more often: P2 > P4 > P3 > P1, the risky rats showed a clear preference for the disadvantageous option, most often the option with maximal uncertainty: P3 > P4 > P2 > P1. Once again animals sampled the various options throughout the task.

We found a main effect of chronic ropinirole on choice in the first week of treatment, but this did not depend on rats’ choice strategy, and this effect disappeared in subsequent weeks, suggesting that chronic ropinirole destabilized choice behaviour on this task without affecting choice of a specific option (Figure 4.4 A-B; Table 4.3; week 1- drug treatment: $F_{1,36} = 5.13, p = 0.030$; option x drug treatment: $F_{3,108} = 0.93, p = 0.431$, NS; Figure 4.4 C-F; drug treatment x choice strategy: $F_{1,36} = 0.51, p = 0.478$, NS; week 2-4- drug treatment: all Fs < 2.22, all $p$s > 0.145, NS; option x drug treatment: all Fs < 0.74, all $p$s > 0.533, NS; drug treatment x choice strategy: all Fs < 0.36, all $p$s > 0.551, NS). Not finding a specific change in any of the options on this task suggests that the effect of ropinirole was small in magnitude and most likely distributed between the four various options. We also found an effect of ropinirole on decision making when comparing the first week of ropinirole to baseline behaviour that did not depend on the option chosen (Figure 4.4; drug treatment week 1 vs baseline: drug treatment group x time point: $F_{1,36} = 5.55, p = 0.024$; ropinirole-treated: time point: $F_{1,18} = 18.03, p < 0.001$; time point x option: $F_{3,54} = 1.06, p = 0.375$, NS; saline-treated: time point: $F_{1,18} = 2.22, p = 0.153$, NS; time
point x option: \( F_{3.54} = 0.18, \ p = 0.908, \ NS \); drug treatment week 2-4 vs baseline: drug treatment
group x time point: all Fs < 1.67, all ps > 0.205, NS; ropinirole-treated: time point: all Fs < 3.85, all ps > 0.065, NS; time point x option: all Fs < 1.14, all ps > 0.343, NS; saline-treated: time
point: all Fs < 1.38, all ps > 0.256, NS; time point x option: all Fs < 0.97, all ps > 0.414, NS).
Figure 4.4 Choice behaviour on the cued rGT during chronic ropinirole

Figure 4.4. Chronic ropinirole destabilized choice behaviour on the cued rGT. Data is shown pre-drug treatment and during drug treatment, but this did not depend on baseline preference. Percent choice of the various options in saline (A) and in the ropinirole treated animals (B). Percent choice of the various options in the optimal decision-makers (C-D), and in risk-preferring rats (E-F). Data shown are mean ± SEM.
Table 4.3  Choice behaviour on the cued rGT during chronic ropinirole

<table>
<thead>
<tr>
<th></th>
<th>Time point</th>
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<td>Option</td>
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<td>Week 2</td>
<td>Week 3</td>
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<td></td>
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<td>Saline</td>
<td>P1</td>
<td>5.82 ± 2.14</td>
<td>6.48 ± 2.98</td>
<td>5.95 ± 2.40</td>
<td>7.31 ± 2.96</td>
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<td>P2</td>
<td>16.38 ± 4.86</td>
<td>18.52 ± 5.29</td>
<td>15.88 ± 4.85</td>
<td>17.97 ± 5.78</td>
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<td>P3</td>
<td>50.82 ± 9.54</td>
<td>47.00 ± 9.92</td>
<td>46.27 ± 9.73</td>
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<tr>
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<td>P4</td>
<td>27.02 ± 7.69</td>
<td>28.00 ± 7.94</td>
<td>31.91 ± 8.05</td>
<td>32.48 ± 7.96</td>
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<tr>
<td>Risk-preferring</td>
<td>P1</td>
<td>0.81 ± 0.60</td>
<td>0.98 ± 0.56</td>
<td>0.98 ± 0.53</td>
<td>0.79 ± 0.69</td>
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<td>71.85 ± 8.79</td>
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<td>78.70 ± 6.47</td>
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<td>P3</td>
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<td>4.26 ± 2.54</td>
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<td>24.55 ± 6.45</td>
<td>18.10 ± 5.34</td>
<td>18.27 ± 5.00</td>
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<td>3.52 ± 1.24</td>
<td>9.75 ± 2.78</td>
<td>14.69 ± 4.74</td>
<td>10.10 ± 3.84</td>
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<td>decision-makers</td>
<td>P2</td>
<td>13.83 ± 3.12</td>
<td>19.48 ± 3.57</td>
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<td>15.43 ± 4.40</td>
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<td>P3</td>
<td>50.49 ± 8.84</td>
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<td>26.58 ± 7.84</td>
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<td>P1</td>
<td>2.96 ± 1.63</td>
<td>4.74 ± 1.71</td>
<td>5.42 ± 3.12</td>
<td>7.49 ± 4.98</td>
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<tr>
<td>Risk-preferring</td>
<td>P2</td>
<td>76.81 ± 4.61</td>
<td>71.09 ± 6.57</td>
<td>69.52 ± 12.70</td>
<td>62.96 ± 11.12</td>
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<td>P3</td>
<td>10.75 ± 5.38</td>
<td>14.18 ± 5.97</td>
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Once again, we investigated whether individual rats responded to the drug. In this study, we looked at rats’ behaviour during the first week of ropinirole administration since the effect of the drug appeared more robust at this time point. In this group of rats, 10 rats (7 from the risky group), could arguably form a responder group, but when looking at these two groups separately, both the responder and non-responder group showed a change in behaviour from baseline. However, only the responder group showed a change when compared to the saline group, and this did not depend on choice strategy (baseline vs drug-treatment- timing x group: $F_{1,18} = 4.79$, $p = 0.042$; responder only, time point: $F_{1,8} = 16.67, p = 0.004$; non-responder only, time point: $F_{1,8} = 6.66, p = 0.033$; responders vs non-responders: $F_{1,16} = 0.01, p = 0.916$, NS; responder vs saline: $F_{1,26} = 5.37, p = 0.029$; non-responder vs saline: $F_{1,26} = 2.88, p = 0.102$, NS; analysis by choice strategy; all Fs < 2.50, all $ps > 0.133$, NS; number of responders/non-responders and conservative/risky: $\chi^2(1) = 0.267, p = 0.606$, NS).

**Non-choice variables.** Chronic ropinirole dramatically increased premature responding in all rats performing the cued rGT, regardless of individual differences in choice strategy. Although this effect diminished in magnitude over time, it remained significant for the duration of drug administration (Figure 4.5 A-B; drug treatment- week 1: $F_{1,36} = 62.68, p < 0.001$; all other weeks: Fs > 14.58, all $ps < 0.001$; Figure 4.5 C-F; drug treatment x choice strategy: all Fs < 0.97, all $ps > 0.331$, NS). In contrast to the standard rGT, risk-preferring rats made more premature responses on the cued rGT at baseline compared to optimal decision-makers, but this difference between the groups was no longer evident during treatment with ropinirole (baseline-choice strategy: $F_{1,36} = 4.70, p = 0.037$; choice strategy x drug treatment: $F_{1,36} = 0.64, p = 0.430$, NS; week 1-4- choice strategy: all Fs < 3.43, all $ps > 0.072$ choice strategy x drug treatment: all Fs < 0.97, all $ps > 0.331$, NS). Similar to data from the uncued version of the task, choice
behaviour at baseline did not predict the number of premature responses made following chronic ropinirole (Figure 4.6; ropinirole treated only: $F_{1,18} = 0.18, p = 0.676, R^2 = 0.010, \text{NS}$).
Figure 4.5. Chronic ropinirole potently increased premature responding on the cued rGT, regardless of baseline choice strategy. Data is shown pre-drug treatment and during drug treatment. Percent premature responding in the saline treated rats (A), and in ropinirole treated rats (B). Premature responses made in the optimal decision-makers (C-D), and in risk-preferring animals (E-F). Data shown are mean ± SEM.
Figure 4.6 Prediction between score on the cued rGT and premature responses

Figure 4.6. Regression analysis between the score for choice on the cued rGT and premature responding during chronic ropinirole. As per the uncued rGT, score on the cued rGT did not predict premature responses induced by chronic ropinirole. Data shown are mean for each rat.
Data values for all other non-choice variables are given in Table 4.4. Ropinirole decreased the number of choice omissions, an effect that was evident from the second week of drug treatment and did not depend on individual choice strategy (drug treatment- week 1: $F_{1,36} = 2.04, p = 0.162, \text{NS}$; week 2: $F_{1,36} = 11.70, p = 0.002$; week 3: $F_{1,36} = 4.910, p = 0.033$; week 4: $F_{1,36} = 6.911, p = 0.013$; choice strategy: all Fs < 2.103, all $p$s > 0.156, NS; drug treatment x choice strategy: all Fs < 2.032, all $p$s > 0.163, NS). Chronic ropinirole also decreased the latency to choose an option, once again independent of whether rats were classified as risk-preferring or optimal decision-makers (drug treatment- baseline: $F_{1,36} = 0.875, p = 0.356, \text{NS}$; week 1: $F_{1,36} = 8.76, p = 0.005$; week 2: $F_{1,36} = 13.27, p = 0.001$; week 3: $F_{1,36} = 15.62, p < 0.001$; week 4: $F_{1,36} = 19.80, p < 0.001$; choice strategy: all Fs < 1.87, all $p$s > 0.180, NS; drug treatment x choice strategy: all Fs < 1.29, all $p$s > 0.263, NS). Similar to the uncued rGT, risk-preferring rats were faster at collecting reward at baseline, and this effect remained unchanged during treatment with ropinirole (choice strategy- baseline: $F_{1,36} = 19.61, p < 0.001$; all other Fs > 5.41, all $p$s < 0.026; drug treatment: all Fs < 3.48, all $p$s > 0.070, NS; drug treatment x choice strategy: all Fs < 1.82, all $p$s > 0.186, NS). Optimal decision-makers again performed more trials, once again likely due to the more frequent occurrence of longer punishments arising from risky rats’ preference for the disadvantageous options (baseline- choice strategy: $F_{1,36} = 41.81, p < 0.001$; all other Fs > 8.134, $p$s < 0.007). However, ropinirole treatment decreased the number of trials performed during the first week of treatment, regardless of animals’ choice preference, likely due to the dramatic increase in premature responses observed during this time point. This effect disappeared by week 2 of chronic administration (drug treatment: week 1: $F_{1,36} = 10.16, p = 0.003$, all other Fs < 3.36, all $p$s > 0.075, NS; drug treatment x choice strategy: all Fs < 3.45, all $p$s > 0.072, NS).
Table 4.4  Other measurements on the cued rGT during chronic ropinirole

<table>
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<tr>
<th></th>
<th>Premature Responses</th>
<th>Choice Latency</th>
<th>Collection Latency</th>
<th>Omission</th>
<th>Trials</th>
</tr>
</thead>
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<tr>
<td><strong>Saline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk-preferring</td>
<td>24.03 ± 3.24</td>
<td>1.69 ± 0.24</td>
<td>0.59 ± 0.04</td>
<td>1.32 ± 0.49</td>
<td>62.07 ± 3.06</td>
</tr>
<tr>
<td>Optimal decision-makers</td>
<td>16.55 ± 3.40</td>
<td>1.52 ± 0.09</td>
<td>0.86 ± 0.08</td>
<td>2.24 ± 0.65</td>
<td>81.80 ± 15.34</td>
</tr>
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<td><strong>Ropinirole</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk-preferring</td>
<td>61.37 ± 3.72</td>
<td>0.99 ± 0.10</td>
<td>0.61 ± 0.04</td>
<td>0.68 ± 0.56</td>
<td>42.24 ± 2.54</td>
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<tr>
<td>Optimal decision-makers</td>
<td>61.21 ± 4.23</td>
<td>0.68 ± 0.12</td>
<td>0.80 ± 0.03</td>
<td>0.08 ± 0.08</td>
<td>57.96 ± 5.36</td>
</tr>
</tbody>
</table>
4.4 Discussion

In these studies, we showed that chronic ropinirole had a destabilizing effect on choice behaviour in rats performing the cued rGT, but not in those performing the uncued version of the task. In contrast to the strong effect of ropinirole in increasing choice of uncertainty in the rBT (Tremblay et al, 2016), we found a more subtle, but significant effect of ropinirole on choice of the cued rGT that was not specific to any of the various options chosen. Similar to results from the rBT, this effect did not depend on animals’ baseline preference for the advantageous or disadvantageous options. In addition, chronic ropinirole increased the number of premature responses made on both versions of the rGT, but this effect was more potent and long lasting on the cued version of the task. Consistent with observations on the rBT, ropinirole decreased the number of omissions made, potentially suggesting greater motivation to engage in the task during ropinirole treatment.

Although we observed a subtle destabilizing effect of chronic ropinirole on choice on the cued rGT, this effect was much smaller compared to that observed on the rBT, and was not apparent on the uncued version of the task. This may speak to the intrinsic differences between the rBT and the rGT. The concept of loss on decision-making tasks in rodents has been a topic of controversy (see Winstanley et al, 2016a). Although in human gambling tasks, subjects may lose accrued money on loss trials, it is difficult to model loss in the same way using food reward in rodents, the reward being consumed immediately following delivery. On the rGT and cued rGT, loss is modeled both by a time out punishment, and by a lost opportunity to receive rewards, with choice of the disadvantageous options leading to longer time out punishments and therefore less sugar rewards over time. In contrast, there is no time out period on the rBT, and also no real penalty or disadvantage in choosing one option over the other, the two options being
equal in terms of the number of sugar rewards that can be earned over time. This difference between the tasks alters the neurobiological regulation of task performance (Winstanley and Floresco, 2016b). For example, amphetamine has different effects on choice, depending on whether explicit penalties are present or absent. Whereas amphetamine increases choice of riskier probabilistic options when omission of reward is the only negative outcome (St Onge and Floresco, 2009), this drug decreases choice of the risky options on the rGT, in which a time out penalty accompanies the omission of reward (Zeeb et al, 2009). From this perspective, it is plausible that ropinirole, which primarily works on the dopamine system, leads to different effects on the rBT compared to the rGT because of this difference in the representation of loss between the tasks.

In addition, the rGT, like the IGT used clinically, may show a higher level of decision-making complexity that may not be replicated in binary decision-making tasks such as in the rBT. In the rGT, rats decide between four options, therefore a decrease in choice of one option may increase choice of various other options, diminishing our capacity to find a difference in choice of one specific option. In contrast, choice of the safe lever is necessarily the inverse of choice of the uncertain option on the rBT, particularly given the low level of omissions. Not only do these two tasks model and represent different types of decision-making and gambling behaviours, but the inherent complexity associated with choice on the rGT may increase the number of brain regions and neurotransmitter systems recruited into regulation of the choice process, such that impacting one circuit has less behavioural impact due to more distributed control (Cocker et al, 2015; Floresco et al, 2008; Winstanley et al, 2016a; Winstanley et al, 2006; Winstanley et al, 2010a).
Somewhat in support of this suggestion, performance of the rBT and the rGT does appear to be controlled by different neural circuitry, with performance of the rBT relying on the dorsal striatum-OFC circuit, and the rGT relying on the ventral nucleus accumbens (NAc)-basolateral amygdala (BLA)-medial prefrontal cortex (mPFC) circuit (i.e. Barrus et al, 2016a; Tremblay et al, 2014; Zeeb et al, 2011; Zeeb and Winstanley, 2013). These differences may account for both the lack of effect of ropinirole on the rGT, the more subtle effect on choice observed on the cued rGT, and the pronounced increase in motor impulsivity on both rGT variants. The BLA is strongly interconnected with the NAc and the PFC, and forms part of the ventral, mesolimbic reward pathway (Cardinal et al, 2002; Cardinal et al, 2003). In previous studies using the uncued rGT, lesions of the BLA performed prior to task acquisition delays adoption of the optimal strategy on the task, whereas lesioning this area once the strategy has been learned leads to increased disadvantageous choices (Zeeb et al, 2011). In marked contrast, BLA lesions had no effect on performance of the rBT (Tremblay et al, 2014). The BLA therefore has a stronger involvement in regulating choice on the rGT, likely due to the more prominent concept of loss involved (Canessa et al, 2013; De Martino et al, 2010; Sokol-Hessner et al, 2013; Yacubian et al, 2006). Furthermore, the increase in risk taking shown in PD patients with ICDs appears to be unrelated to loss aversion (Voon et al, 2011a).

In contrast, the OFC, which is strongly interconnected with the dorsal striatum, may be more strongly involved in performance of the rBT. In support for this hypothesis, inactivation of the OFC leads to increase in preference for uncertainty in wager-sensitive rats on the rBT (Barrus et al, 2016a), somewhat similar to the effect of ropinirole on this task, whereas OFC lesions and inactivations performed after animals have acquired the rGT do not affect decision-making patterns on task. In a previous study, pramipexole was shown to increase activity in the
lateral OFC during negative feedback, suggesting that this drug may impair reward processing in the OFC (van Eimeren et al, 2009). Therefore, ropinirole may have greater effects on OFC-dependent tasks.

As mentioned, there is much inconsistency in clinical studies about whether patients with PD are impaired on the IGT (Bentivoglio et al, 2013; Euteneuer et al, 2009; Pignatti et al, 2012; Rossi et al, 2010). Since the IGT requires many neural networks and more distributed control, individual differences in the progression of the disease, along with the differential effect the addition of DRT drugs may have on these potentially damaged networks, may explain the inconsistencies observed in the clinical population. Nonetheless, previous work also suggests that the dopamine system may not be primarily involved in mediating choice on the uncued rGT, with dopaminergic drugs targeting principally the D2 receptors having a limited effect on choice behaviour on this task (Baarendse and Vanderschuren, 2012; Barrus et al, 2016b; Zeeb et al, 2009). Therefore, although DRT may increase tonic levels of dopamine in PD patients and may increase reward seeking and impulsivity possibly through the ventral striatum, the involvement of other systems may explain why they can leave intact the ability to learn and discriminate amongst various contingencies, such as in the rGT (Dagher et al, 2009). As such, the negative findings we observe on choice of the rGT may not be entirely unexpected. In contrast, risky choice on the cued, but not uncued, rGT can be enhanced by a D3 receptor agonist, and attenuated by a D3 antagonist. Choice therefore appears more dopamine dependent on this variant of the rGT. This may explain why ropinirole had some effect on decision-making patterns on task. However, clearly the effects of chronic ropinirole were far more subtle than a bolus injection of highly selective D3 agents, and did not match the effects of a D3 agonist. Although ropinirole is a D3-preferring ligand, this drug also has high affinity and efficacy at D2
receptors. Dual activation of both receptor subtypes may therefore produce distinct effects from activation of D₃ receptors alone.

Chronic ropinirole increased premature responses, a measure of motor impulsivity, on both versions of the rGT, an effect that was exacerbated on the cued rGT. Also, contrary to a previous study which found a correlation between disadvantageous decision-making on the rGT and impulsivity (Barrus et al, 2015), we found no such relationship. It has previously been shown that a high level of this form of impulsivity, as measured using the 5CSRT, predicted more rapid acquisition of cocaine self-administration (Dalley et al, 2007) and a more addiction-like engagement with drug-taking (Belin et al, 2008). As to the mechanism through which ropinirole may be amplifying motor impulsivity, other D₂ family agonists generally do not produce this effect when given acutely. For example, the D₂/₃ agonist quinpirole decreased the number of premature responses on the rGT and 5CSRT (Winstanley et al, 2010b; Zeeb et al, 2009). While a low dose of the D₃ agonist PD128907 significantly increased this measure of impulsivity on the uncued version of the rGT, no change was observed on the cued rGT (Barrus et al, 2016b). Nevertheless, high motor impulsivity on the 5CSRT has generally been associated with amplified dopamine release in the NAc: the ability of amphetamine to promote premature responding can be attenuated by direct administration of dopamine antagonists into the NAc. Ropinirole may therefore be amplifying premature responding through its actions in this brain region.

D₃ receptors are concentrated in the NAc (Bouthenet et al, 1991; Levesque et al, 1992), and highly impulsive rats exhibit lower D₂/₃ receptor availability in this brain region (Dalley et al, 2007). In the latter study, the ligand used did not differentiate between D₂ and D₃ receptors, or between pre-synaptic vs post-synaptic receptor expression. However, the NAc has been
involved in the control of impulsivity. Specifically, this region receives excitatory glutamate and dopamine from the PFC and VTA, respectively, amongst other nuclei. Contribution from the PFC depends on dopaminergic modulation within the NAc. The PFC, which sends excitatory glutamate that then activate inhibitory GABAergic neurons within the NAc is modulated by presynaptic D₂ autoreceptors on glutamatergic terminals within the NAc. Stimulation of D₂ autoreceptors therefore inhibits the ability of the PFC to influence NAc inhibitory action. Theoretically, stimulating D₂ autoreceptors in the ventral striatum with D₂/₃ agonists may therefore reduce the inhibitory control the prefrontal cortex exerts on the NAc and increase impulsivity (Grace et al, 2007).

In the rBT, the proportion of rats that showed an increase in preference of the uncertain outcome in response to ropinirole was higher than the incidence of DRT-induced ICD in the clinical population, and argued that the repeated exposure to uncertain outcomes may have sensitized the dopamine system and therefore could account for the higher proportion of animals responding to the drug (Singer et al, 2012; Zack et al, 2014). Similarly, a greater proportion of rats performing the cued vs uncued rGT preferred P3, the option with a 50:50 chance of reward, and therefore, with maximal uncertainty. Sensitization of the dopamine system following exposure to uncertain outcomes, or cues paired with uncertain outcomes, could therefore account for the stronger effect of ropinirole on both premature responding and decision making in the cued rGT. D₃ receptors in the ventral striatum have been implicated in learning the association between cues and reward, and in sensitization (Beninger and Banasikowski, 2008; Richtand, 2006; Wolf, 1998). These receptors, which can be expressed on D₁ receptors in models of PD, have also been involved in behavioural sensitization to L-DOPA in rats (see Sokoloff et al, 2001). In addition, D₃ receptors have been implicated in the motivation to take drugs of abuse
and in drug seeking behaviours, as well as in the reaction to cues associated to drugs of addiction (Sokoloff and Le Foll, 2017). From this perspective, although speculative, chronic stimulation of D3 receptors with ropinirole could have led to the increase in impulsivity observed on both versions of the rGT. In addition, repeated experience with the cues in the cued rGT could have further sensitized this system, and explain the magnified effect on impulsivity observed on the cued version of the task.

Chronic ropinirole also transiently decreased omissions and the latency to choose an option on the cued rGT, while only omissions were affected on the standard version of the task. We have recently shown that chronic ropinirole invigorates performance of a rat slot machine task, another model of gambling-related behaviour, as indicated by faster latencies to respond and increased trials completed (Cocker et al, 2017). Decreased omissions may similarly suggest increased engagement with the task, similar to the increase in motivation to gamble observed in those with iatrogenic GD caused by DRTs. In support for this hypothesis, dopaminergic drugs (including DRTs and L-DOPA) increased the speed of decision-making and therefore increase reflection impulsivity in PD patients with ICDs, without affecting performance of the Stroop task (Voon et al, 2010).

Ropinirole works both on D2 and D3 receptors. Although studies points to a role of D3 receptors in the ventral striatum in cue-reward association and in impulsivity, the dorsal striatum, which is rich in D2 receptors may also be involved. A recent study showed a relationship between D2/3 receptor levels and impulsivity following cocaine administration in both the dorsal and ventral striatum (Caprioli et al, 2013). In addition, a study in humans showed an increase in D3 receptor binding in the ventral striatum and slight decrease in D2 in the dorsal striatum of polydrug users (Boileau et al, 2012). Future studies should determine the level of D2 and D3 receptor
levels in both of these areas at baseline, as well as following chronic ropinirole in rats performing the rGT and cued rGT. However, D₂ and D₃ receptors can also form heterodimers, and activation of these receptors by pramipexole and ropinirole may play a functionally unique role in mediating ICDs following DRT treatment (Scarselli et al., 2001).

This study suggests that adding cues to the rGT triggers a destabilizing effect on choice following chronic ropinirole, and potentiates an increase in premature responses observed on both versions of the tasks. The differential effect of ropinirole on the rGT compared to the rBT may help us understand the mechanisms involved in the deficits observed in PD patients developing ICDs following initiation of DRTs. In addition, sensitization of the dopamine system with the addition of cues associated with wins on the task may help us understand the importance these cues have in GD and in DRT-induced ICDs.
Chapter 5: Involvement of the GSK3β pathway in mediating the effect of ropinirole on the rBT

Inhibition of GSK3β does not attenuate the increase in choice of uncertainty observed following chronic ropinirole administration on the rBT

5.1 Introduction

Given that DRTs are agonists at D_2-like receptors, it would seem reasonable to hypothesize that individuals who are most at risk for iatrogenic ICDs following chronic administration of these drugs may express greater numbers of these receptors in key nodes within the affective frontostriatal loop, a brain circuit responsible for mediating impulsive or risky behaviours. High levels of striatal D_2 receptors have been observed in PD patients using positron emission topography (PET) during early stages of the disease, a time when the risk for developing ICDs in response to DRT is higher (Dagher et al, 2009; Nikolaus et al, 2009; Voon et al, 2007), yet a recent report suggests that PD-ICD patients do not exhibit greater levels of D_2 or D_3 receptors as compared to PD patients without ICDs (Payer et al, 2015a). In animals performing the rBT, lower levels of D_{2/3} receptors in the dorsal striatum was associated with higher levels of wager-sensitivity, a behaviour considered irrational, and potentially associated with vulnerability to addiction (Cocker et al, 2012a). Nonetheless, the relationship between receptor levels and gambling behaviour remains a topic under debate.

Alternatively, differences in the manifestation of DRT-induced ICDs could reflect differences in the intracellular signaling pathways activated by ligand-binding at D_2 receptors. As a G-protein coupled receptor (GPCR), the intracellular signaling pathway predominantly associated with D_2 receptor activation involves a reduction in cyclic adenosine monophosphate
(cAMP) production and inhibition of protein kinase A (PKA), leading to decreased phosphorylation of the dopamine- and cAMP-regulated phosphoprotein (DARPP) at threonine 34. However, D<sub>2</sub> receptors also signal through a G-protein/cAMP-independent Akt/GSK3β pathway mediated by β arrestin-2 (Beaulieu et al., 2011; Beaulieu et al., 2005; Li et al., 2011). D<sub>2</sub> activation leads to inhibition of Akt1 phosphorylation by β arrestin-2 through protein phosphatase 2A (PP2A), leading to elevation in GSK3β levels. GSK3β has been shown to be involved in multiple neurological and psychiatric disorders such as drug addiction, schizophrenia and bipolar disorder in humans, as well as in hyperdopamine-dependent behaviours in animals (Beaulieu et al., 2005; Beaulieu et al., 2004; Peterson et al., 2015b). It is therefore plausible that activation of GSK3β signaling may contribute to DRT-induced ICDs. Furthermore, β arrestin-mediated signaling pathways have a slower onset, and a more prolonged duration than G-protein mediated GPCR signaling. G-protein mediated responses have a rapid onset and desensitization, whereas the desensitization mechanism for β arrestin-mediated cell signaling is unclear. It has therefore been suggested that the Akt/GSK3β pathway can dominate during prolonged, continuous stimulation of dopamine D<sub>2</sub> receptors, and may thus reflect long-lasting/tonic changes in dopamine concentrations or chronic agonist administration (Beaulieu et al., 2011; Beaulieu et al., 2004).

A growing body of literature suggests that selective GSK3β inhibitors can have a variety of beneficial effects, including reducing inflammation in response to infections, protecting organs against injury, and reversing deficits in cognition caused by a range of insults (Dash et al., 2011; Dugo et al., 2006; Franklin et al., 2014; Kwon et al., 2014; Verhees et al., 2013; Willi et al., 2013). GSK3β inhibitors can attenuate cocaine- and amphetamine-induced hyperactivity and sensitization, behavioural phenomena that likewise depend critically on elevated dopamine
release in the striatum (Enman and Unterwald, 2012; Kalinichev and Dawson, 2011; Miller et al., 2009, 2010). Therefore, it is suggested here that this drug class might reverse ropinirole-induced increases in risky choice on the rBT. In partial support of this hypothesis, lithium and valproic acid, mood stabilizers that inhibit GSK3α/β among their multiple mechanisms of action, have both been suggested as treatments for PD, and case studies have shown that valproic acid may reduce symptoms of dopamine dysregulation syndrome in L-DOPA medicated PD patients (Epstein et al., 2014; Sriram et al., 2013; Wang et al., 2013). However, whether these drugs are appropriate for treating PD patients is uncertain. For example, lithium may also increase dyskinesia symptoms when combined with L-DOPA, and its effect on the primary motor symptoms of PD is controversial (Coffey et al., 1984; Dalen and Steg, 1973). While lithium may reduce gambling problems in bipolar disorder patients it can actually create PD-like symptoms in this population. Such PD-like symptoms have also been observed in the elderly population treated with valproic acid (Masmoudi et al., 2006; Silver and Factor, 2013). In addition, although valproic acid may help in controlling symptoms of ICDs in PD patients on DRTs, it may worsen PD symptoms in patients also taking L-DOPA (Hicks et al., 2011). Therefore, although lithium and valproic acid could potentially be useful in treating symptoms of ICDs, they may interact negatively with the dopamine medication used to treat PD, and the safety of valproic acid in the elderly is questionable.

Given that lithium and valproic acid may be acting by multiple diverse mechanisms, it is currently unknown whether GSK3β inhibition is beneficial in reducing DRT-induced ICDs, or whether this action of the drug is responsible for the adverse effects encountered when interacting with other PD treatments or the pathology of PD itself. In addition, although studies have evaluated the potential benefit of other pharmacological treatments to counteract DRT-
induced ICDs in PD patients, their efficacy, or the mechanism by which they could procure beneficial effects remain elusive (see Ramirez-Zamora et al, 2016 for review). It would therefore be of enormous utility if we could determine whether specific GSK3β inhibition specifically can ameliorate ropinirole’s effects on decision making on the rBT. Therefore in this study, we replicated our finding that chronic ropinirole increased choice of uncertainty in rats performing the rBT. We then tested whether chronic inhibition of GSK3β with SB 216763 (3[2,4-Dichlorophenyl]-4-[1-methyl-1H-indol-3-yl]-1H-pyrrole-2,5-dione), an arylinolmaleamide, ATP-competitive GSK3 inhibitor, could attenuate or block this effect on decision making. We also measured levels of key proteins in dopaminergic signaling pathways following chronic ropinirole treatment to better understand how they may be related to behaviour on the rBT.

5.2 Additional methods

5.2.1 Task training

Subjects (n = 31) were trained to perform the rBT as described in previous chapters (see Figure 2.2; total sessions to behavioural stability: 55).

5.2.2 GSK3β inhibitor Latin Square challenge

Once behavioural stability was achieved, an initial pilot study (n = 16) to determine a dose of the ATP-competitive GSK3β inhibitor SB 216763 (Tocris, Bristol, United Kindom) for the chronic study was performed. Doses of SB 216763 ranging from 0.25 to 10 mg/kg have been tested acutely and chronically in rodents. Previous studies indicate that 5 mg/kg increases brain levels of phosphorylated GSK3β at serine 9 (pGSK3β9; the inactive form of GSK3β) in rats, and repeated administration prevents development of behavioural sensitization to psychostimulant
drugs (Enman et al., 2012; Miller et al., 2009, 2010). However, the effects of this compound on any operant tasks have yet to be reported. We therefore first confirmed that acute administration of SB 216763 does not impair performance of the rBT. Three doses of SB 216763 (1.25, 2.5, 5.0 mg/kg) plus vehicle were prepared fresh daily and administered intraperitoneally (IP) 30 mins prior to testing according to a diagram-balanced Latin Square design with three doses and a vehicle as follows: A = vehicle, B = smallest dose, C = middle dose, D = highest dose (for doses A-D: ABCD, BDAC, CADB, DCBA as per Cocker et al., 2012a). SB 216763 and vehicle were administered on a three-day schedule which started with a baseline testing day. A dose of the drug or the vehicle was administered the following day. The third day constituted of a day off testing allowing for washing off the drug. During this SB 216763 challenge, the GSK3β inhibitor was administered at a time-point sufficiently ahead of training on the rBT to allow the drug’s activity to peak. SB 216763 was dissolved in 2% dimethyl sulfoxide (DMSO), 1% tween 20, 5% polyethylene glycol 400, and diluted to a volume of 10 ml/kg with distilled water. The solution was continuously stirred to avoid the drug falling out of solution. Rats were then trained on the rBT for two weeks following the Latin Square challenge in order to allow for drug washout and return to baseline. Two rats were euthanized following a bad reaction to a previous compound solvent tested.

5.2.3 Osmotic pump implantation

Once baseline was re-established following the SB 216763 Latin Square challenge, animals were divided into two groups of rats and implanted with an osmotic pump delivering either 5 mg/kg/day of ropinirole hydrochloride (n = 19) or 0.09% saline solution (n = 10) for 28 days. The rats that underwent the Latin Square experiment were proportionally distributed
between these groups (ropinirole group: 9 from the Latin Square experiment; saline: 4 from the Latin Square experiment). Based on previous data shown in Chapter 3, about 2/3rds of rats will show significant increases in choice of the uncertain option by the end of the first week of ropinirole treatment that will continue to increase until the reservoir is empty. We therefore biased the allocation of rats in order to ensure sufficient numbers (6-7/10) of rats were likely to exhibit an increase in risky choice in response to ropinirole per pharmacological challenge condition. The dose of ropinirole used in this experiment was identical to that in previous studies (see Chapter 3-4; Cocker et al, 2017; Tremblay et al, 2016). One rat was euthanized following osmotic pump implantation due to a bad reaction to ropinirole.

5.2.4 Chronic GSK3β inhibitor challenge

Two weeks following implantation of the osmotic pump, and once a ropinirole-induced increase in uncertain choice was significant on the rBT, rats were again divided into two equivalent groups and received one daily IP injection of either 5 mg/kg SB 216763 (n = 14; 9 ropinirole, 5 saline, 7 from the Latin Square experiment), or vehicle (n = 14; 9 ropinirole, 5 saline, 6 from the Latin Square experiment) 30 minutes prior to testing for 10 consecutive days. Again, consideration was taken to distribute the rats who underwent the Latin Square challenge equally between the groups. Since all the doses of SB 216763 in the Latin Square challenge were behaviourally silent, we started the chronic SB 216763 challenge at the highest dose of 5 mg/kg, which was previously shown to inhibit GSK3β (Dash et al, 2011). However, since this dose did not attenuate increase in preference for uncertainty in response to chronic ropinirole in the first 5 days of administration, we increased the dose of the GSK3β inhibitor to 7.5 mg/kg for the remaining 5 days of chronic ropinirole/SB 216763. At the end of the 28 day period of
chronic ropinirole, which coincided with the end of chronic SB 216763, rats were humanely euthanized by live decapitation.

5.2.5 **Ex vivo analysis**

Here we analysed brains from the healthy rats who performed the rBT in Chapter 3, as well as those from the GSK3β experiment for comparison. Rats in the original rBT experiment were euthanized following a 4 weeks washout period from ropinirole whereas those in the GSK3β experiment were euthanized while still exposed to pharmacological challenge. We also could compare whether our dose of the GSK3β inhibitor SB 216763 was efficient in elevating levels of phosphorylated GSK3β or decreasing levels of GSK3β. Tissue samples from the nucleus accumbens (NAc) and dorsal striatum were harvested and flash frozen. Western blotting was then used to determine levels of key proteins in dopaminergic signaling pathways: dopamine D1 and D2 receptors, cyclic adenosine monophosphate (cAMP) regulated phosphoprotein with molecular weight 32 kDa (DARPP), phosphorylated DARPP at threonine 34 (pDARPP\textsubscript{34}), cAMP response element binding protein (CREB), glycogen synthase kinase-3beta at serine 9 (GSK3β\textsubscript{9}), and β-tubulin as control.

5.2.6 **Western Blotting**

Using a RIPA buffer (SDS, 10%; IGEPAL, 1%; Sarkosky, 0.5%; NaCl, 150 mM; Tris base, 50 mM; and standard protease inhibitor), tissue was homogenized in a 3:1 buffer-to-tissue ratio. Protein concentration and purity were assessed using a NanoDrop spectrophotometer. Remaining homogenized tissue was stored at -20°C before sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) assay. Samples were thawed at room temperature and combined with Laemmli sample buffer using a 1:1 ratio (BioRad, Mississauga,
Ontario, Canada). Samples were loaded onto polyacrylamide gel in running buffer (19 Tris glycine solution) and run at 110 V for 1:30 h. A protein ladder was included in each gel as a control for protein movement. Gels were run again through a resolving layer at 110 V to complete protein movement. Following SDS-PAGE, proteins on gels were transferred to a polyvinylidene fluoride membrane. Once proteins had been transferred, blots were blocked in Odyssey blocking buffer (Li-Cor) solution. Gels were then incubated overnight with primary and secondary antibodies for the following proteins: dopamine D₁ and D₂ receptors, cAMP, DARPP-32, pDARPP₃₄, CREB, and β-tubulin as control (see Table 5.1 for specific primary antibodies and dilution). Following incubation, blots were imaged using a LiCor spectrophotometer (Lincoln, NE, USA), and fluorescence of each blot was quantified and normalized for statistical analysis.
<table>
<thead>
<tr>
<th>Protein</th>
<th>Primary antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>D₁ receptor</td>
<td>1: 200 dilution purified rabbit polyclonal primary antibody, Santa Cruz Biotechnology, cat. no. SC-14001</td>
</tr>
<tr>
<td>D₂ receptor</td>
<td>1:200 dilution purified rabbit polyclonal primary antibody, Santa Cruz Biotechnology, cat. no. SC-9113</td>
</tr>
<tr>
<td>DARPP-32</td>
<td>1:1000 dilution purified mouse monoclonal primary antibody, BD Biosciences, cat. no. 611520</td>
</tr>
<tr>
<td>pDARPP-32 (Thr-34)</td>
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</tr>
<tr>
<td>CREB</td>
<td>1:1000 dilution purified mouse monoclonal primary antibody, Cell Signaling Technology, cat. no. 9104</td>
</tr>
<tr>
<td>GSK3β</td>
<td>1:1000 dilution purified rabbit polyclonal primary antibody, Cell Signaling Technology, cat. no. 9315</td>
</tr>
<tr>
<td>β-tubulin (control)</td>
<td>1:10,000 dilution purified mouse monoclonal primary antibody, Millipore, cat. no. 05-661</td>
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</table>
5.2.7 Data analysis

Analyses were conducted using SPSS (version 22, SPSS/IBM, Chicago, USA). Behaviour was assessed at baseline, following the Latin Square challenge, and throughout the 28 days of ropinirole delivery and SB 216763 chronic administration. During the Latin Square challenge, data were analyzed using drug (4 levels, 3 doses plus saline) as a within-subject factor in a repeated-measure ANOVA. During the chronic ropinirole experiment, data were analysed in weekly bins of 5 daily sessions with session (5 levels, sessions 1-5) as a within-subjects factor and “ropinirole” group (2 levels, ropinirole vs saline) as a between subjects factor. With the addition of chronic SB 216763, we also added “inhibitor” group (2 levels, SB 216763 vs vehicle) as another between subject factor. The Greenhouse-Geisser correction was applied whenever the assumption of sphericity was violated. As for previous experiments using the rBT, the key dependent variable was the percent choice of the uncertain lever, analysed as a within-subjects factor across bet size (3 levels, 1-3 sugar pellets). Individual rats were also classified as wager-sensitive or wager-insensitive based on their choice of the uncertain option as a function of bet size, and this between subjects factor was also included in all analyses (wager-sensitivity, 2 levels).

5.3 Results

5.3.1 GSK3β Latin Square challenge

Choice behaviour. As observed in previous studies using the rBT, wager-insensitive rats (n = 18) sampled fairly equally from both the safe and uncertain levers, regardless of the bet size in play (bet size: $F_{2,34} = 1.40, p = 0.260, \text{NS}$), whereas wager-sensitive animals (n = 11) drastically decreased their preference for the uncertain reward as the bet size increased (bet size:
$F_{2.20} = 19.08, p < 0.001)$. SB 216763 did not affect choice behaviour, regardless of rats’ baseline preference for the safe or uncertain option (Figure 5.1 A; dose: $F_{3.36} = 0.67, p = 0.575, \text{NS}$; Figure 5.1 B-C; dose x wager-sensitivity: $F_{3.36} = 0.75, p = 0.527, \text{NS}$; dose x bet size: $F_{6.72} = 1.55, p = 0.175, \text{NS}$).
Figure 5.1  Choice behaviour on the rBT during the Latin Square with SB 216763

Figure 5.1. Various doses of SB 216763 did not affect decision making on the rBT. Data shown is choice of the uncertain lever for the various doses in all rats (A), in wager-sensitive (B), and in wager-insensitive rats (C). Data shown are mean ± SEM
Non-choice variables. SB 216763 also did not affect any other task parameters, irrespective of rats’ baseline behaviour (Table 5.2; choice latency, collection latency, choice omission and hole omission; dose, dose x wager-sensitivity, dose x bet size: all $F_s < 2.63$, all $p_s > 0.106$, NS).
Table 5.2 Other measurements on the rBT during the Latin square challenge

<table>
<thead>
<tr>
<th>Bet Size</th>
<th>Choice Latency-Safe</th>
<th>Choice Latency-Uncertain</th>
<th>Collection Latency-Safe</th>
<th>Collection Latency-Uncertain</th>
<th>Choice Omission</th>
<th>Hole Omission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.08 ± 0.21</td>
<td>1.77 ± 0.14</td>
<td>0.43 ± 0.05</td>
<td>0.40 ± 0.03</td>
<td>0.00 ± 0.00</td>
<td>3.11 ± 0.63</td>
</tr>
<tr>
<td>2</td>
<td>1.84 ± 0.13</td>
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<td>0.41 ± 0.03</td>
<td>0.38 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>1.22 ± 0.43</td>
</tr>
<tr>
<td>3</td>
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<td>1.86 ± 0.10</td>
<td>0.39 ± 0.03</td>
<td>0.37 ± 0.02</td>
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<td>4.56 ± 0.73</td>
</tr>
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</tr>
<tr>
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<td>1.84 ± 0.12</td>
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<tr>
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<td>3.67 ± 0.67</td>
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<td>5.0 mg/kg</td>
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</tr>
<tr>
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<td>0.40 ± 0.01</td>
<td>0.38 ± 0.02</td>
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<td>3.60 ± 1.47</td>
</tr>
<tr>
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<td>2.19 ± 0.13</td>
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<td>0.41 ± 0.01</td>
<td>0.35 ± 0.01</td>
<td>0.00 ± 0.00</td>
<td>1.80 ± 0.20</td>
</tr>
<tr>
<td>3</td>
<td>1.89 ± 0.06</td>
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<td>0.39 ± 0.01</td>
<td>0.35 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>3.20 ± 0.58</td>
</tr>
</tbody>
</table>
5.3.2 Chronic ropinirole

Choice behaviour. As observed in the previous experiment in Chapter 3 in healthy rats performing the rBT, chronic ropinirole increased choice of the uncertain lever, regardless of animals’ baseline preference for the safe or uncertain option, and this was evident during the second week of ropinirole (Figure 5.2 A-B; Table 5.3; week 1- ropinirole group: $F_{1,24} = 2.44, p = 0.131$, NS; week 2- ropinirole group: $F_{1,24} = 5.127, p = 0.033$; ropinirole x bet size, Figure 5.2 D-E,G-H; ropinirole x wager-sensitivity: all $F$s < 2.01, all $p$s > 0.169, NS). Once again, the ropinirole-induced increase in preference for uncertainty was evident both when the saline group was compared to the ropinirole group, and also when each drug treatment group was compared to its respective baseline prior to osmotic pump surgery (ropinirole- week 2 vs baseline: ropinirole x time point: $F_{1,25} = 13.06, p = 0.001$; ropinirole-treated: $F_{1,17} = 15.67, p = 0.001$; saline-treated: $F_{1,8} = 2.92, p = 0.126$, NS).
Figure 5.2 Choice behaviour on the rBT during chronic ropinirole and SB 216763

Figure 5.2. Chronic ropinirole increased choice of the uncertain lever on the rBT, an effect not attenuated by SB 216763. Data is shown pre-drug treatment, during drug ropinirole, and during SB 216763. Percent choice of the uncertain lever in all rats (A-C). The effect of ropinirole was observed regardless of whether rats were wager-sensitive (D-F), or wager-insensitive (G-I). Choice behaviour in the responder and non-responder rats (J-L). There was no true non-responder rats in this group of animals. Data shown are mean ± SEM.
<table>
<thead>
<tr>
<th>Table 5.3 Choice behaviour on the rBT during ropinirole/SB 216763</th>
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</thead>
<tbody>
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<td><strong>Time point</strong></td>
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<td>---</td>
</tr>
<tr>
<td><strong>Bet Size</strong></td>
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<tr>
<td>Saline</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Saline</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Ropinirole</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
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<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>
As per the previous chapters, additional analyses to determine whether a subgroup of rats drove the effect of ropinirole were performed. These analyses identified that some rats (n = 10; wager-sensitive: n = 3, wager-insensitive: n = 7) showed a stronger response to ropinirole and significantly increased their preference for uncertainty compared to the saline rats (Figure 5.1 L; ropinirole- week 2 vs baseline, responders vs non-responders: time point x responder group: \(F_{1,14} = 10.16, p = 0.007\); responders only, time point: \(F_{1,8} = 67.23, p < 0.001\); saline vs responder: \(F_{1,18} = 6.04, p < 0.024\); analysis by wager-sensitivity; all Fs < 1.32, all ps > 0.283, NS; number of responders and wager-sensitive/wager-insensitive: \(\chi^2(9) = 12.22, p = 0.201, NS\)). However, although the group of “non-responder” rats (n = 8; wager-sensitive: n = 3, wager-insensitive: n = 5) was not significantly different when compared to the saline group, they nonetheless showed an increase in choice of uncertainty when compared to their respective baseline behaviour and also did not statistically differ from the responder rats (ropinirole- week 2 vs baseline, non-responders only, time point: \(F_{1,6} = 12.31, p < 0.013\); saline vs non-responder: response: \(F_{1,16} = 2.33, p < 0.147, NS\); responders vs non-responders: \(F_{1,14} = 1.09, p < 0.313, NS\); analysis by wager-sensitivity; all Fs < 0.50, all ps > 0.505, NS; number of responders/non-responders and wager-sensitive/wager-insensitive: \(\chi^2(1) = 0.11, p = 0.999, NS\).

Non-choice variables. Data values for non-choice variables measured are given in Table 5.4. Rats receiving ropinirole made less hole omissions while performing the rBT compared to the rats receiving saline, an effect that was not present prior to chronic ropinirole and continued in the second week of drug administration. This effect also did not depend on rats’ preference for the uncertain or safe option (ropinirole- baseline: \(F_{1,25} = 1.93, p = 0.177, NS\); ropinirole week 1: \(F_{1,24} = 6.21, p = 0.020\); week 2: \(F_{1,24} = 6.15, p = 0.021\); ropinirole x wager sensitivity: all Fs < 1.24, all ps > 0.277, NS). Ropinirole did not affect any other behavioural parameter in this group.
of rats (choice and collection latencies, and choice omission: drug treatment group: all $F_s < 3.85$, all $p_s > 0.065$, NS; ropinirole x wager-sensitivity: all $F_s < 2.15$, all $p_s > 0.159$, NS).
### Table 5.4 Other measurements on the rBT during ropinirole/SB 216763

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<th>Bet Size</th>
<th>Choice Latency-Safe</th>
<th>Choice Latency-Uncertain</th>
<th>Collection Latency-Safe</th>
<th>Collection Latency-Uncertain</th>
<th>Choice Omission</th>
<th>Hole Omission</th>
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<td>Saline-Vehicle 1</td>
<td>2.30 ± 0.27</td>
<td>2.25 ± 0.10</td>
<td>0.45 ± 0.04</td>
<td>0.60 ± 0.14</td>
<td>0.00 ± 0.00</td>
<td>3.42 ± 1.39</td>
</tr>
<tr>
<td>2</td>
<td>2.18 ± 0.08</td>
<td>2.33 ± 0.09</td>
<td>0.43 ± 0.03</td>
<td>0.57 ± 0.14</td>
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<td>1.89 ± 0.45</td>
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<td>2.02 ± 0.09</td>
<td>0.41 ± 0.04</td>
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</tr>
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<td>2.38 ± 0.63</td>
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<td>1.85 ± 0.15</td>
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<td>0.00 ± 0.04</td>
<td>5.04 ± 1.50</td>
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<td>0.39 ± 0.04</td>
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<td>0.37 ± 0.04</td>
<td>0.35 ± 0.01</td>
<td>0.00 ± 0.00</td>
<td>3.25 ± 1.61</td>
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</table>
5.3.3 Chronic GSK3β inhibition

Choice behaviour. Chronic administration of SB 216763 did not reduce the increase in uncertain choice in response to ropinirole at either the 5 mg/kg or the 7.5 mg/kg dose regardless of wager-sensitivity, or whether rats were in the responder or non-responder group (Table 5.3; 5 mg/kg dose of SB 216763- inhibitor group: $F_{1,20} = 0.01$, $p = 0.944$, NS; ropinirole group: $F_{1,20} = 7.33$, $p = 0.014$; ropinirole group x inhibitor group: $F_{1,20} = 0.01$, $p = 0.907$, NS; responder group x inhibitor group: $F_{1,10} = 0.35$, $p = 0.569$, NS; Figure 5.2 C; Table 5.3; 7.5 mg/kg dose of SB 216763- inhibitor group: $F_{1,20} = 0.02$, $p = 0.894$; ropinirole group: $F_{1,20} = 10.77$, $p = 0.004$; ropinirole group x inhibitor group: $F_{1,20} = 0.01$, $p = 0.906$, NS; Figure 5.2 L; responder group x inhibitor group: $F_{1,10} = 0.08$, $p = 0.789$, NS; inhibitor x bet size, Figure 5.2 F,I; inhibitor x wager-sensitivity: all $Fs < 0.601$, all $ps > 0.487$, NS). We also did not see an effect of chronic GSK3β inhibition when comparing behaviours to week 2 of ropinirole treatment, or to rats’ original baseline prior to osmotic pump surgery for any of the two SB 216763 doses used (time point: all $Fs < 1.54$, all $ps > 0.229$, NS; time point x inhibitor group: all $Fs < 1.12$, all $ps > 0.303$, NS; time point x inhibitor group x responder group: all $Fs < 0.54$, all $ps > 0.480$, NS).

Non-choice variables. Data values for non-choice variables measured are given in Table 5.4. Rats receiving SB 216763 made more hole omissions during the 5 mg/kg drug administration, an effect that was not previously present, and which disappeared during subsequent challenge with 7.5 mg/kg. This increase in omissions also did not depend on rats baseline preference for the safe or uncertain option or ropinirole group (5 mg/kg dose of SB 216763- inhibitor group: $F_{1,15} = 5.41$, $p = 0.031$; inhibitor x wager-sensitivity: $F_{1,15} = 0.31$, $p = 0.582$, NS; inhibitor group x ropinirole group: $F_{1,15} = 0.93$, $p = 0.347$, NS; baseline- inhibitor group: $F_{1,15} = 3.03$, $p = 0.097$, NS; 7.5 mg/kg dose of SB 216763- inhibitor group: $F_{1,15} = 4.09$, $p$
Statistical analyses indicated an interaction between the SB 216763 treatment group and choice latency of the uncertain lever at different bet sizes during the 5 mg/kg administration (inhibitor x bet size: \(F_{2,30} = 11.64, p < 0.001\)). However, when the effect of the drug was analysed at the different bet sizes separately, we detected no significant effects (bet size 1-3: all \(Fs < 2.47\), all \(ps > 0.129\), NS). The significant interaction term from the main ANOVA likely indicates that the drug was having differential effects at the various bet size, e.g. decreasing the latency to choose a lever at bet size 2 and 3 in drug treated animals, but that none of these effects were strong enough to drive an effect at any one bet size. A similar effect was observed for the latency to collect a safe reward during the 7.5 mg/kg administration, whereupon the SB 216763 treated animals were slower to collect rewards at bet size 2 and vehicle treated animals were slower at the smaller bet size (inhibitor x bet size: \(F_{2,30} = 6.57, p = 0.008\)). However, once again this effect was not strong enough to show a reliable change in collection latency at any one bet size (bet size 1-3: all \(Fs < 1.19\), all \(ps > 0.290\), NS). Treatment with SB 216763 did not affect any other behavioural parameters at any of the two doses tested (choice and collection latencies, choice and hole omissions: inhibitor group: all \(Fs < 1.10\), all \(ps > 0.314\), NS; inhibitor x wager-sensitivity: all \(Fs < 3.44\), all \(ps > 0.081\), NS; inhibitor group x ropinirole group: all \(Fs < 3.69\), all \(ps > 0.072\), NS; inhibitor x bet size: all \(Fs < 1.652\), all \(ps > 0.223\), NS).

5.3.4 Western blotting

*Original rBT experiment.* One hypothesis in the original rBT study was that animals who showed a more robust behavioural response to a D\(_{2/3}\) receptor agonist would exhibit greater D\(_2\) receptor expression in the brain area responsible for mediating its behavioural effect. We have previously observed a positive correlation between D\(_2\) receptor density in the dorsal striatum and preference for the uncertain outcome across bet size, hence our focus on dopamine signaling in
striatal regions (Cocker et al., 2012a). We therefore performed western blot on tissue samples harvested from this group of rats. We observed significantly higher levels of D2 receptors in dorsal striatal samples taken from rats classified as responders to ropinirole on the rBT as compared to saline-treated controls (Figure 5.3 A; D2: t18 = 2.52, p = 0.022). This elevation in D2 receptors was not observed in non-responders (D2: t13 = 1.53, p = 0.150, NS). D2-like receptors are slow metabotropic receptors coupled to inhibitory G-proteins. Activation of D2 receptors can decrease cyclic adenosine monophosphate (cAMP) production, thereby inhibiting protein kinase A (PKA), leading to a decrease in phosphorylation of DARPP at threonine 34 (pDARPP34). However, we only observed significantly lower levels of pDARPP34 and CREB in rats treated with ropinirole that did not exhibit a behavioural response to the drug (Figure 5.3 B; pDARPP: t10.04 = 2.93, p = 0.015; CREB: t11.89 = 2.60, p = 0.023; all other t’s < 0.40, all ps > 0.696, NS). As such, this signaling mechanism is unlikely to contribute to the behavioural effects of ropinirole on the rBT. D2 receptors also signal through a G-protein/cAMP independent β arrestin-2 mediated AKT/GSK3β pathway, which may dominate under hyperdopaminergic conditions as mimicked by chronic D2/3 agonist administration (Beaulieu et al., 2011; Li et al., 2011). In partial support of a role for this pathway in mediating ropinirole’s effects on the rBT, we observed a trend toward higher levels of GSK3β in the dorsal striatum of rats classified as responders that was not observed in the non-responder group (Figure 5.3 B; responders vs saline: t6 = 2.23, p = 0.067; non-responders vs saline: t2.17 = 0.86, p = 0.474, NS). No other analyses revealed significant or trend-level results in either the dorsal or ventral striatum (Figure 5.3 C-D; DARPP, pDARPP, CREB: all ts < 1.66, all ps > 0.112, NS).
Figure 5.3. Relative protein expression assessed by Western blotting in rats from the original rGT. Chronic ropinirole significantly increased expression of D2 receptors (A) and we observed a trend toward increased expression GSK3β in the striatum of responder rats (B). We also observed lower levels pDARPP and CREB in the non-responder rats in the striatum (B). Protein levels were not altered in the nucleus accumbens (NAc; C-D). Signal intensities for D1, D2, DARPP, pDARPP (threonine 34), CREB, and GSK3β, normalized to β-tubulin, are expressed as percentage control levels and representative blots are shown with corresponding β-tubulin bands underneath. Sal: saline treated, NR: non-responders, R: responders to the drug. Data shown are mean ± SEM.
**GSK3β experiment.** Since the changes in protein levels from the original rBT was observed in the dorsal striatum, analysis of tissue samples from the ropinirole/GSK3β experiment focussed on protein levels in this area. Contrary to our expectation, SB 216763 did not globally reduce levels of GSK3β in the dorsal striatum (Figure 5.4 B; GSK3β: $t_{19.21} = 1.70, p = 0.105, \text{NS}$). However, rats receiving ropinirole, regardless of whether they subsequently received the GSK3β inhibitor or the vehicle, or were in the responder or non-responder group, showed a significant increase in GSK3β in this area, an effect that was not significant in the saline-vehicle controls (ropinirole-vehicle: $t_{13} = 3.05, p = 0.009$; ropinirole-inhibitor: $t_{12} = 2.541, p = 0.026$; saline-inhibitor: $t_{8} = 1.557, p = 0.158, \text{NS}$; responder: $t_{13} = 2.45, p = 0.029$; non-responder: $t_{11} = 2.66, p = 0.022$). This lack of differentiation between the responder and non-responder rats may speak of the lack of a truly non-responder group in the behavioural analysis. In addition, we saw a trend towards elevated D2 expression in the rats as a group who received the GSK3β inhibitor (Figure 5.4 A; D2: $t_{26} = 1.99, p = 0.057, \text{NS}$). However, rats who received ropinirole and the vehicle showed a slight but significant reduction in D2 receptors in this area, an effect that was interestingly not seen in those receiving ropinirole and the inhibitor (ropinirole-vehicle: $t_{13} = 3.87, p = 0.002$; ropinirole-inhibitor: $t_{12} = 1.07, p = 0.307$). We did not observe other significant results for any of the other proteins analysed (DARPP, pDARPP, CREB, pCREB: all $t$s $< 1.59$, all $p$s $> 0.136$, NS).
Figure 5.4. Relative protein expression assessed by Western blotting in the chronic GSK3β inhibition experiment. SB 216763 did not reduce levels of GSK3β in the dorsal striatum. Rats receiving ropinirole, regardless of subsequently receiving SB 216763, showed increase in GSK3β in this area (B). Rats who received ropinirole and the vehicle showed a slight reduction in D2 receptors in this area, an effect not seen in those receiving ropinirole and the inhibitor (A). There was also a trend towards elevated D2 expression in the rats as a group who received SB 216763 (A). Signal intensities for D2, DARPP, pDARPP (threonine 34), CREB, and GSK3β, normalized to β-tubulin, are expressed as percentage control levels and representative blots are shown with corresponding β-tubulin bands underneath. S-V: saline-vehicle treated, S-G: saline-SB 216763 treated, R-V: ropinirole-vehicle, R-G: ropinirole-SB 216763 treated. Data shown are mean ± SEM.
5.4 Discussion

Here we showed that chronic ropinirole leads to a strong and reliable effect on increasing preference for uncertainty in rats performing the rBT, and that this effect could not be blocked by treatment with the GSK3β inhibitor SB 216763. In Chapter 3, we showed that some rats could be classified as responders or non-responders based on their increased preference for the uncertain option during chronic ropinirole treatment (Tremblay et al, 2016). When assessing the level of key proteins potentially involved in D2-receptor signaling using Western blotting, we found a trend towards higher levels of GSK3β, yet no change in cAMP response element-binding protein (CREB) or phosphorylated DARPP at threonine 34 (pDARPP34), in rats who increased their preference for uncertainty following chronic ropinirole, indicative of predominant activation of the D2-mediated β-arrestin signaling pathway. In contrast, ropinirole-treated animals that did not shift their choice preference in response to the drug showed lower levels of pDARPP34 and CREB in this area, yet no change in GSK3β, suggesting that D2-mediated PKA-dependent signaling prevailed. Collectively, the recruitment of GSK3β signaling pathways in responder rats, but not in non-responders, pointed to a mechanism through which ropinirole may have biased preference towards uncertainty on the rBT.

Converging lines of evidence had suggested that GSK3β may be a mechanistic driver of the behavioural effects of ropinirole given its involvement in psychiatric disorders such as drug addiction, schizophrenia and bipolar disorder, as well as its role in hyperdopamine-depandant behaviours (Beaulieu et al, 2005; Beaulieu et al, 2004; Peterson et al, 2015). Prior studies had showed that dopaminergic drugs like cocaine activate the Akt/GSK3β pathway (Miller et al, 2014; Perrine et al, 2008), and SB 216763 reduces hyperactivity induced by dopaminergic drugs in mice (Enman et al, 2012; Miller et al, 2009, 2010). Repeated low doses of pramipexole were
also shown to activate this pathway (Ma et al., 2016). In addition, knock-in mice with deregulated phosphorylation of GSK3β showed increased susceptibility to the locomotor effect of amphetamine (Polter et al., 2010). In further evidence for involvement of this pathway, valproate and lithium, both of which are potent inhibitor of GSK3β, have shown at least partial success in treating GD (Beaulieu et al., 2005; Hollander et al., 2005; Pallanti et al., 2002). Therefore, we hypothesised that inhibition of the Akt/GSK3β pathway target kinase GSK3β might block or attenuate the increase in uncertain choices induced by ropinirole.

We therefore replicated the effect of chronic ropinirole on decision making on the rBT in another group of healthy rats and attempted to ameliorate the behavioural effect of ropinirole with the GSK3β inhibitor SB 216763. Acute administration of various doses (1.25, 2.5, 5.0 mg/kg) of SB 216763 alone did not affect decision making on the rBT. However, we replicated the effect of chronic ropinirole in increasing preference for uncertainty in this new group of rats, but surprisingly, were unable to block or reduce this effect by chronic inhibition with SB 216763. In contrast to rats from the previous studies, we could not differentiate a true non-responder group since, although not significantly different from the saline treated group, this groups of rats did show a more moderate increase in choice of uncertainty from baseline and was also not significantly different from the responder group. Somewhat consistent with earlier findings, ropinirole decreased hole omissions, suggesting stronger engagement with the task. GSK3β inhibition transiently increased hole omissions and collection latency after a safe lever choice, and moderately reduced choice latency of the risky lever. Analysis of the tissue samples from the dorsal striatum in this new group of rats showed a more global increase in levels of GSK3β, in ropinirole-treated rats regardless of whether rats received the inhibitor SB 216763 or the vehicle. It is therefore tempting to conclude that chronic ropinirole engaged the Akt/GSK3β
pathway leading to increase in GSK3β. Visual inspection of the data suggest that the saline-SB 216763 rats also had higher levels of this kinase, but critically this was not significant, and likely reflects natural variation in GSK3β levels between individual animals.

The discrepancies between brain tissue levels of proteins from the experiment in Chapter 3 and the present experiment are interesting. Animals in Chapter 3 underwent 4 weeks of washout from ropinirole prior to the brains being harvested for tissue analysis. In comparison, animals in the present study were euthanized while still exposed to both ropinirole and SB 216763. The Akt/GSK3β pathway mediated by β arrestin-2 has a slow onset, and a more prolonged duration than G-protein mediated GPCR signaling. This pathway was suggested to be predominantly activated during prolonged and continuous stimulation of dopamine D2 receptors such as with chronic ropinirole or repeated administration of pramipexole, and may thus lead to long-lasting/tonic changes (Beaulieu et al, 2011; Beaulieu et al, 2004; Ma et al, 2016). The GSK3β kinase is constitutively active and inactivated through phosphorylation of serine 9 residue of the regulatory amino-acid terminal domain (Beaulieu et al, 2009). Similar to a previous study that showed a sustained effect of ropinirole on decreasing pGSK3β, which would lead to elevation of GSK3β levels, that lasted for 4 weeks following termination of ropinirole treatment (Cocker et al, 2017), we also saw a trend towards increased GSK3β following ropinirole treatment in responder rats 4 weeks after termination of ropinirole. This lasting effect of chronic ropinirole on protein levels was observed even past the behavioural return to baseline in both studies. From this perspective, it appears that the sustained elevation of GSK3β and D2 receptors observed in the original rBT study is not unexpected and may be consistent with activation of this pathway during chronic ropinirole in the responder rats.
Surprisingly, chronic SB 216763 administration alone did not reduce levels of GSK3β; levels of this kinase were elevated in rats that received ropinirole, regardless of whether they subsequently received the GSK3β inhibitor or vehicle. Given that chronic administration of the GSK3β inhibitor failed to noticeably reduce levels of GSK3β, or attenuate the behavioural effects of ropinirole, combined with the fact that we replicated an increase in GSK3β signaling within the dorsal striatum in ropinirole-treated rats from both the current behavioural experiment and the rats used in Chapter 3, it therefore remains plausible that ropinirole alters decision-making via this mechanism.

The reason for the lack of reduction in GSK3β when SB 216763 was administered independently is unclear. Here, SB 216763 was administered in rats compared to mice in previous studies mentioned above. The rate at which SB 216763 is metabolized may well differ between the two species (Martignoni et al, 2006a; Martignoni et al, 2006b). However, doses used in this study were similar, or even higher than those used in other studies that showed a decrease in GSK3β levels in rat brain, and were effective in counteracting conditioned place preference and addiction-related behaviours in this species (Huang et al, 2015; Wickens et al, 2017). However, we did not find this drug easy to dissolve and, due to animal care restrictions, were not allowed to dissolve the drug in 100% DMSO for chronic IP injections such as seen in acute challenges in other studies. Although we tried to dissolve the drug in a lesser percentage and used various solvents, we were unable to completely dissolve the drug to a stable solution, so the drug was injected as a suspension. This may have dampened the absorption rate, diffusion through the blood brain barrier, or metabolism of SB 216763 and thereby restricted its action on intracellular signaling. Nonetheless, another study injected this drug as a suspension at doses similar to the ones used here and observed a decrease in locomotor activity in DAT-KO mice.
(Beaulieu et al, 2004). Ideally, we would have assayed brain concentrations of SB 216763 to conclusively determine whether we failed to achieve adequate brain penetrance. However, we do not have the equipment or expertise for such analyses.

GSK3 is a constitutively active kinase and present with two isoforms, α and β. It is regulated positively by phosphorylation of threonine 21, but negatively via phosphorylation of serine 9. In addition, reduction in GSK3 levels can lead to autoregulation through increase in the N-terminal phosphorylation of GSK3, which can also be regulated through GSK3-dependant protein phosphatase-1 complex. Furthermore, the two isoforms seem to have different functions, but can influence regulation of each other in complex ways (Liang and Chuang, 2007; Zhang et al, 2003). SB 216763 is a small molecule that acts to inhibit both GSK3α and β. It is unclear if chronic dosing in our study could lead to complex autoregulation mechanisms that may ultimately explain why we did not see a decrease in GSK3β levels, and hence a failure to block the behavioural effects of ropinirole on the rBT. As alternate GSK3β inhibitors become available, or other compounds that may selectively target this signaling cascade (Barak et al, 2016; Gao et al, 2015; Peterson et al, 2015a; Peterson et al, 2015b), it may be possible to more specifically determine whether ropinirole elevates preference for the uncertain outcome via this signaling cascade. Given that GPCR kinases such as activation of β arrestins can trigger independent signaling cascades and initiate desensitization processes in other intracellular mechanisms (see Andresen, 2011 for review), chronic ropinirole may trigger DRT-induced ICDs through activation of the Akt/GSK3β pathway and by desensitization of the cAMP/PKA intracellular pathway. Rather than using pharmacological ligands, this question may be better answered using molecular biology techniques.
Whereas D\textsubscript{2} receptor levels were elevated in ropinirole-treated rats in Chapter 3, animals receiving chronic ropinirole and vehicle in the current study showed a decrease in D\textsubscript{2} receptors, an effect that appeared to be blocked in animals receiving the GSK3\textbeta{} inhibitor. We also saw a trend towards increased D\textsubscript{2} receptors in the SB 216763 treated-rats. It is unclear here why we observed differences in D\textsubscript{2} expression following chronic treatment with ropinirole in the two studies. Once again, it could be argued that the difference in the two experimental timelines may account for this differential effect on dopamine receptors. However, as per the lasting effect of GSK\textbeta{} in a previous study (Cocker \textit{et al}, 2017), changes in D\textsubscript{2} receptors following dopaminergic drugs have been observed to last three months to a year following abstinence in humans and primates (Nader \textit{et al}, 2006; Volkow \textit{et al}, 1993). This suggests that the change observed here may be long-lasting and not due to the difference in experimental timeline. Nonetheless, another study has found no difference in D\textsubscript{2} receptors following 14 days of chronic pramipexole in rat striatum, but an increase in D\textsubscript{3} in this area (Tokunaga \textit{et al}, 2012). We did not measure levels of striatal D\textsubscript{3} receptors, but this receptor may play a role in the effect of ropinirole on the rBT, or in mediating the involvement of the Akt/GSK3\textbeta{} pathway. In support for this hypothesis, mice lacking the D\textsubscript{3} receptor required a higher dose of dopaminergic drugs to activate Akt-mediated signaling, suggesting that these receptors may enhance the D\textsubscript{2} receptor response to dopaminergic drugs (Beaulieu \textit{et al}, 2007). In addition, a variant of the dopamine D\textsubscript{3} receptor, the AA genotype of DRD3 p.S9G, but not the D\textsubscript{2} variant DRD2 Taq 1A, which has been associated with impulsivity and addictive disorders (Comings, 1994; Noble \textit{et al}, 1993; Smith \textit{et al}, 1992), was also associated with risk to develop ICDs in a population of PD patients (Lee \textit{et al}, 2009). Also, the dopamine D\textsubscript{3} receptors in the substantia nigra may be involved in gambling severity and
Impulsivity (Boileau et al., 2013), and drugs acting predominantly at this receptor have been suggested to increase the risk to develop ICDs (Seeman, 2015).

Alternatively, co-administration of ropinirole and the GSK3β inhibitor may have interacted in intricate ways to block a potential effect of ropinirole on D₂ receptors. Emerging evidence suggests that activation of heterodimer receptors such as the D₂/₃ receptors may play a functionally unique role that may differ from activation of either D₂ or D₃ receptor alone (Scarselli et al., 2001). In addition, activation of one site of the heterodimer may negatively modulate binding at the other site of the heterodimer receptor, a process termed “allosterism”, and has been described for various receptors subtypes such as the dopamine D₂ receptor (Rocheville et al., 2000). It has been proposed that this allosterism may enhance functional activity and allow for highly selective or unexpected action of drugs. In contrast to other drugs acting as D₂/₃ agonists, ropinirole and pramipexole showed amplified potency at D₂/D₃ heterodimers as compared to monomers, an effect that may be uniquely related to their relationship to the development of ICDs in patients (see Maggio et al., 2009). The idea that inconsistent drug effects may be attributed to parallel or consecutive downstream signaling following receptor binding, determined by individual variation in the intracellular molecular environment, is not novel (Galandrin et al., 2007; Rajagopal et al., 2010; Urban et al., 2007). Therefore, although highly speculative, it is possible that co-administration of SB 216763, by interacting with intricate intracellular pathways which may already be dynamically regulated by ropinirole through these heterodimer receptors, may explain why a change in D₂ receptors was not seen when both SB 216763 and ropinirole were co-administered. In support for this hypothesis, it appears that all rats receiving ropinirole showed an increase in GSK3β level, but
the change in D₂ receptor expression did not occur when ropinirole and SB 216763 were co-administered in this group of rats.

Regardless, the relationship between D₂ receptor levels and ICDs in PD is still unclear. Although high levels of D₂ receptors have been observed in patients using positron emission topography (PET) during early stages of PD, a time when the risk for developing ICDs in response to DRT is higher (Dagher et al., 2009; Nikolaus et al., 2009; Voon et al., 2007), a recent report suggests that PD-ICD patients do not exhibit greater levels of D₂ or D₃ receptors as compared to PD patients without ICDs (Payer et al., 2015a). We also cannot determine from the current dataset whether individual difference in levels of D₂ expression are evident prior to drug treatment and influence the response to ropinirole, or whether the differences in receptor expression are caused by ropinirole itself. Measurement of D₂ receptor levels before vs after ropinirole treatment using micro-PET imaging could help to resolve this issue.

To add to this complexity, prolonged exposure of ropinirole or pramipexole to cells that express heterodimer D₁/D₃ receptors, leads to supersensitivity of D₁ receptor activated adenylyl cyclase (AC) (Maggio et al., 2009). Here we did not see an effect of chronic ropinirole on D₁ receptors or on the PKA-dependent pathway, except in rats who did not increase their preference for uncertainty in response to ropinirole in Chapter 3, suggesting little involvement of these receptors in the behavioural effect of ropinirole. In addition, D₂ and D₃ receptors are inhibitory, and can be autoreceptors located on pre-synaptic dopamine neurons, as well as post-synaptic receptors located on inhibitory interneurons and medium spiny neurons in the striatum (Ford, 2014; Seamans et al., 2004). The Western blot analyses conducted here do not allow us to decipher if the changes observed in our studies reflect changes at the pre- vs post-synaptic level, nor do they allow us to ascertain whether there were any changes in internalization vs. insertion.
of receptors at the cell membrane. However, stimulation of autoreceptors in the ventral striatum are believed to reduce the excitatory influence, and therefore inhibitory control, from the prefrontal cortex (Grace et al, 2007). Although we did not see any evidence of intracellular signaling changes in the ventral striatum in the samples we analysed, it may be worth confirming this null effect in future studies.

Alternatively, the ability of ropinirole to modulate decision making may be impacted by other neurotransmitter systems or signaling pathways. It has been shown that sustained administration of pramipexole, which is similar in action to ropinirole, can increase spontaneous firing of neurons in systems other than the dopamine system such as norepinephrine and serotonin and desensitized both D2/3 and 5-HT1A autoreceptors (Chernoloz et al, 2009). The serotonin (5-HT) system has been heavily implicated in the regulation of impulse control and decision making, including in the problematic behaviours seen in PD on DRT (Crockett, 2009; Crockett et al, 2012; Crockett et al, 2010; Crockett et al, 2009; Leeman et al, 2013). In rats, global decreases in 5-HT can increase measures of impulsivity (Harrison et al, 1997). Decreased function in the gene coding for the brain 5-HT rate limiting synthesis enzyme, Tph2, is linked to loss of impulse control, and is associated with diseases marked by high impulsivity such as bipolar disorder (BD) and attention deficit hyperactivity disorder (ADHD) (Popova and Kulikov, 2010; Waider et al, 2011). Similarly, R439H Tph2-knock-in (KI) mice, which express a human loss of function Tph2 variant resulting in deficient 5-HT synthesis, show substantial cognitive impairment, as indexed by maladaptive perseveration and poor reversal learning, while also exhibiting high levels of impulsive aggression (Beaulieu et al, 2008; Del'Guidice et al, 2014). In addition, R439H Tph2-KI mice show elevated GSK3β activity, and genetic manipulations that decrease GSK3β in these mice rescue behavioural deficits associated with Tph2-KI (see Beaulieu
et al, 2009). Therefore, the Akt/GSK3β pathway may be strongly implicated in disorders related to the 5-HT system. Whether the effect of ropinirole on desensitization of 5-HT1A receptors or on the 5-HT system more generally could explain the behavioural effect on the rBT is unknown, but may be worthy of future investigation. In partial support for the implication of the 5-HT system in ICDs, chronic administration of the atypical antidepressant mirtazapine, which has 5-HT2c inverse agonist properties amongst other mechanisms, partially reduced pramipexole-induced increase in probabilistic discounting in rats (Holtz et al, 2016).

In conclusion, the experiments in this study undeniably demonstrate that chronic ropinirole has a strong and reliable effect on increasing preference for uncertainty in rats performing the rBT. Although ex vivo analyses of protein levels supported the hypothesis that this behavioural effect may arise through activation of the Akt/GSK3β signaling pathway, our attempt to block ropinirole-induced increases in preference for uncertain outcomes with a GSK3β inhibitor were inconclusive: we were not able to attenuate ropinirole’s effects, but neither could we confirm that the drug used was effective at chronically down-regulating GSK3β signaling. Future studies using gene editing techniques or RNA silencing to inhibit the actions of GSK3β may be required to conclusively test this hypothesis.
Chapter 6: Impact of manipulating dopaminergic tone in the nigrostriatal pathway in the response to ropinirole using DREADDs

Activation of the nigrostriatal pathway with DREADDS mimics the effect of chronic dopamine D_{2/3} agonist ropinirole in increasing gambling-like behaviours on a rodent Betting task, while inhibiting this pathway has no effect

6.1 Introduction

Pathophysiological changes in PD involve a dramatic loss of dopamine neurons in the substantia nigra (SNc) and consequential loss of dopamine terminals in the dorsal striatum, leading to severe movement deficits. Although compensatory mechanisms take place at early stages of the disease that can compensate for the damage, important changes in the system remain, which may compromise its ability to regulate and control behaviour in response to DRT (Wilson et al., 1996; Zigmond et al., 1990). The “overdose” hypothesis was suggested in order to try and explain the development of ICDs such as GD following initiation of DRTs in some patients. According to this theory, DRT treatment would replenish dopamine stores in the deteriorated dorsal striatum, the area where the damage is more severe in PD patients, and restore functioning in the basal ganglia to improve movement. However, the addition of exogenous dopamine with DRT could overwhelm the mostly spared mesolimbic reward system, leading to ICDs (Cools, 2006; Kish et al., 1988). Although this theory seems promising at explaining the role of the dopamine mesolimbic and nigrostriatal pathway in DRT-induced ICDs, the specific mechanism by which they occur is still unknown. Data that we and others have collected also suggest that dopamine depletion is not necessary for the development of ICDs since they can appear in individuals without PD who are prescribed DRTs, and these drugs lead
to increased preference for uncertain outcomes in both healthy and dopamine depleted animals, suggesting that the drug itself may precipitate these side-effects (Rokosik et al, 2012; Tremblay et al, 2016; Voon et al, 2011c).

As mentioned, GD is considered a behavioural addiction in the fifth edition of the DSM-V due to its clinical and neurobiological resemblance to drug addiction (Potenza, 2014). However, important differences between GD and stimulant drug addiction are emerging, further complicating understanding of GD in PD in response to DRTs. For example, reduced D₂ receptor expression and blunted activity in the ventral striatum is a hallmark of substance use disorder (Martinez et al, 2004; Volkow et al, 2007). However, the evidence for such reduced activity or lower levels of D₂ receptor availability in GD is far from definitive. A number of studies found no difference in dopamine receptor levels in the mesolimbic system between individuals with GD and healthy controls, or between PD patients with and without ICDs (i.e. Boileau et al, 2013; Joutsa et al, 2012; Payer et al, 2015b). In addition, studies have also both supported and contradicted the evidence for a sensitized mesolimbic dopamine system in GD (Linnet et al, 2011; Reuter et al, 2005; Steeves et al, 2009).

The dorsal striatum may also play a role in DRT-induced ICDs due to its involvement in the transition to more habitual and compulsive behaviour in addiction (Everitt et al, 2005). In support for this hypothesis, recent studies have shown that increased activity in the dorsal striatum in individuals with GD correlated with increased dopamine release in the ventral striatum and associated with gambling severity (Boileau et al, 2014). Also, increased activity in the dorsal striatum has also been involved in anticipation of monetary rewards in GD patients (van Holst et al, 2012b). As mentioned in the introduction, animal studies showed that D₂/₃ receptor expression in the dorsal striatum was also associated with what can be considered
irrational decision-making in wager-sensitive rats on the rBT (Cocker et al, 2012a). Further suggesting the importance for this area in the potential development of ICDs, our previous study in Chapter 5 showed no difference in protein expression in the ventral striatum, including D2 receptor levels, between rats who increased their choice of uncertainty and those who did not, but instead, we observed an increase in D2 receptors in the dorsal striatum.

As mentioned in the introduction, the lateral orbitofrontal cortex (OFC) has strong connections with the dorsal striatum and is involved in updating the subjective value of rewards. In support for its involvement in cognition, inactivating the lateral OFC in rats increases preference for uncertainty in wager-sensitive rat on the rBT (Barrus et al, 2016a), partially mimicking the effect of ropinirole on this task. In PD with ICD, persistent stimulation of D2 receptors in the basal ganglia indirect pathway may impair learning from negative feedback (Dagher et al, 2009; Frank et al, 2004). Pramipexole was found to increase activity in the lateral OFC activity during negative feedback, suggesting that this drug may impair processing of such events in the OFC (van Eimeren et al, 2009). However, the precise mechanism by which D2/3 agonist drugs act is complex since D2 receptors are located both pre- and post-synaptically, and their action is inhibitory (Ford, 2014; Seamans et al, 2004). Therefore, it would be of great value to investigate the specific contribution of activation and deactivation of the nigrostriatal pathway, which may disturb the functioning of the OFC, and affect decision-making on the rBT. It could also help us understand the mechanism by which dopamine D2/3 agonists drug such as ropinirole can induce ICDs such as GD. In this study, we first assessed the effect of inhibiting the nigrostriatal pathway using DREADDs infused in the SNc prior to chronic ropinirole administration in rats performing the rBT. In a second group of rats, this same pathway was instead excited in an attempt to recreate the effect of chronic ropinirole observed on this task.
6.2 Additional methods and materials

6.2.1 Genotyping

Rats (aged around postnatal day 60) bred in-house used for experiments 1 and 2 of this chapter were genotyped to confirm expression of TH and Cre. After weaning (post-natal day 21), rats were anesthetized and the ear notches obtained for polymerase chain reaction (PCR) analyses. To extract DNA, ear notches were lysed using a buffer solution (50 mM pH 8.0 Tris, 2 mM NaCl, 10 mM EDTA, 1% SDS) and Proteinase K (Invitrogen, part number 25530-015). Extracted DNA was stored at -20°C until PCR was performed. All reactions were performed in 200 μl thin walled PCR tubes with FASTSTART TAQ DNA POL. DNTPACK reaction mix (Roche, cat #4738357001), primers for Cre (forward: 5’-AGA GTA CAC TGT GGG CAG GA-3’; reverse: 5’-GCA AAC GGA CAG AAG CAT TT-3’) or TH (forward: 5’-CGC TTA CCC CGG AAG AAC AA-3’; reverse: 5’-CCA GCA GAG GTA ATG GAA GAG A-3’). Samples were then placed in a thermocycler and underwent standard cycling protocols (95°C 5 mins; then cycled 35 times: 94°C 30 sec, 63°C 30 sec, 72°C 1 min; holding at 72°C for 10 min; infinite holding at 4°C) and subsequently run on an agarose gel at 90V for 40 min to verify genotypic expression.

6.2.2 Experiment 1: Inhibition of the nigrostriatal pathway with DREADDs

Subjects. Animals were 63 transgenic male Long Evans rats from multiple breeder pairs (n = 31 TH:cre positive, n = 32 TH:cre negative; initial weight: 275-300 g) trained to perform the rBT as described in previous chapters (see Figure 2.2; total sessions to behavioural stability: 24).

AAV surgery. Animals were anesthesized with isoflurane (4% induction; ~2.5% maintenance) and given ketoprofen and bupivacaine subcutaneously as systemic and local analgesic. The inhibitory adeno-associated-virus AAV-hSyn-DIO-hM4D(Gi)-mCherry, which
encodes a DREADD coupled to Gi protein inhibiting the phospholipase C (PLC) pathway leading to activation of transfected neurons when CNO is applied, was infused into the SNc (anteroposterior (AP): -5.4 mm; mediolateral (ML): ±2.2 mm; dorsoventral (DV): -7.7 mm). A volume of 1 µL of the AAV virus was infused at a rate of 0.1 µL/min. The injectors were left in place for an additional 10 minutes to allow the infusate to diffuse. Animals were allowed to recover for 8 weeks to allow for expression of the DREADDs. Following the first week of surgical recovery, animals were trained on the rBT to stable baseline behaviour as described in previous chapters.

*Chronic CNO.* Once stable baseline was achieved, animals started receiving twice daily IP injections of 1 mg/kg of clozapine-N-oxide (CNO; Toronto Research Chemicals, North York, Ontario) dissolved into 6% DMSO and saline to a volume of 1 ml/kg. This dose of CNO does not lead to motor impairments as tested in a pilot locomotor study (MacLaren et al, 2016). CNO was administered 30 minutes before testing on the rBT and at least 7 hours elapsed between the 2 injections. Studies have shown that a dose such as the one used in this study was efficient in suppressing about 60% neurons of the ventral pallidum following IP CNO administration (Chang et al, 2015; Mahler et al, 2014). In addition, the average latency for firing suppression following IP CNO was 12 minutes, with, for some cells, more than 70 minutes duration. From the time of onset, rats received chronic CNO, 7 days/week for the duration of the experiment, and throughout osmotic pump implantation surgery and ropinirole/saline delivery.

*Motor assessment: forelimb adjusting step test.* In order to assess both the motor effect of inhibition of dopamine release in the SNc, and the potential therapeutic benefit of ropinirole, animals underwent a forelimb adjusting test 1.5 weeks following the start of CNO administration and 3 weeks following implantation with the osmotic pump delivering either ropinirole or saline.
Similar to the forelimb adjusting test performed in previous studies (Holtz et al, 2016; Rokosik et al, 2012), the experimenter suspended the rear paws and one of the rat forelimb, leaving the rat to support itself on the remaining unrestrained forelimb. The animal was then “dragged” on the unrestrained forelimb for 0.9 m over 5 s in abduction and adduction directions. This motion was repeated for both forelimbs. Measurements were taken twice for each forelimb in each direction and the average number of adjusting steps was determined for each rat.

*Osmotic pump implantation.* Two weeks after the start of chronic CNO, animals were divided into two groups, matched for their baseline performance as described in previous chapters. Rats were then subcutaneously implanted with an osmotic pump delivering either 5 mg/kg/day of ropinirole hydrochloride (n = 40; 20 TH:cre positive, 20 TH:cre negative) or 0.9% saline solution (n = 23; 11 TH:cre positive, 12 TH:cre negative) for 28 days. Training on the rBT resumed 2 days after mini-pump implantation and the effect of inhibition of the nigrostriatal pathway paired with chronic ropinirole was observed. After the 28 days of ropinirole/saline treatment, rats were humanely euthanized by live decapitation.

### 6.2.3 Experiment 2: Excitation of the nigrostriatal pathway with DREADDs

*Subjects.* Animals were 32 transgenic female Long Evans rats from multiple breeder pairs (16 TH:cre positive, 16 TH:cre negative; initial weight: 180-200 g) trained to perform the rBT as described in previous chapters (see Figure 2.2; total sessions to behavioural stability: 27).

*AAV surgery.* Animals underwent surgical procedure for AAV infusion as per experiment 1. The excitatory adeno-associated-virus AAV-hSyn-DIO-hM3D(Gq)-mCherry was infused into the SNc (AP): -5.2mm; mediolateral (ML): ±2.1mm; dorsoventral (DV): -7.5mm). This virus was found to increase neural activity, intracellular synthesis and extracellular dopamine released from transplanted dopamine neurons in an animal model of PD (Dell'Anno et
al, 2014). As per experiment 1, the volume for the AAV virus was 1 µL and infused at a rate of 0.1 µL/min, and the injectors were left in place for an additional 2 minutes for the infusate to diffuse. Animals were also allowed to recover for 8 weeks. Following the first week of surgical recovery, animals were trained on the rBT to stable baseline behaviour as described in previous chapters.

**Chronic CNO.** Once stable baseline behaviour was achieved on the rBT, animals started receiving twice daily IP injections of 1 mg/kg of CNO dissolved in 6% DMSO and saline as per experiment 1. CNO was administered 30 minutes before testing on the rBT to mimic chronic administration of ropinirole, and at least 7 hours elapsed between the 2 injections. Similar to the effect of CNO on inhibitory DREADDs, previous studies have shown that not all cells are affected by CNO administration following infusion of excitatory DREADDs (Vazey and Aston-Jones, 2014b). Rats received chronic CNO, 7 days/week for the duration of the experiment. At the end of chronic CNO (4.5 weeks), rats were humanely euthanized by live decapitation.

### 6.2.4 Immunohistochemistry

At the end of chronic CNO and ropinirole (28 days) in experiment 1, and CNO administration in experiment 2, animals were euthanized by live decapitation and their brain harvested. The caudal half of their brain was put in a solution of 4% phosphate-buffered paraformaldehyde for 24 hours before being stored in a 30% sucrose solution for at least 72 hours in order to confirm expression of hM4D(Gi) and hM3D(Gq) in neurons of the SNc pathway. The rostral half was flash frozen and kept for additional autoradiography analyses not included in this dissertation. The caudal half of the brains was then frozen and sliced at 35 µm on a cryostat. Sections of the SNc were taken and stored as free-floating slices in phosphate buffered saline (PBS).
Brain sections were processed for mCherry and TH immunoreactivity. Tissue was washed in PBS (3 x 5 min) and incubated at room temperature for 1 hour in a PBS blocking solution containing 0.3% triton X and 5% normal goat serum. Sections were then incubated overnight at 4°C in anti-mCherry or anti-TH primary antibodies, washed for 2 x 5 min in PBS, and incubated again overnight with opposite primary antibody. Brain slices were washed with PBS 3 x 5 min followed by a 2 hr incubation period with secondary Alexa 633 (mCherry) or Alexa 488 (TH) antibodies at room temperature. Sections were washed 3 x 5 min in PBS, and incubated in the subsequent secondary antibody for 2 hr. Tissue was washed 3 x 5 min with PBS, mounted onto gelatin-coated glass slides, and cover-slipped with VectaShield anti-fade Mounting Medium (Vector Laboratories, Burlingame, CA, USA). Expression of mCherry and TH was confirmed within the SNc using an AxioZoom V16 microscope (Zeiss, Germany; Figure 6.1).
Figure 6.1 Expression of TH and mCherry in the SNc

Figure 6.1 Micrograph representative of TH and mCherry expression within the SNc with reference to a neuroanatomical rat brain atlas (Paxinos et al, 1998). Micrograph shows expression of TH, mCherry and an overlay of both antibodies in female TH:cre positive (excitatory DREADDs hM3D(Gq)), male TH:cre positive (inhibitory DREADDs hM4D(Gi)), and in a TH:cre negative rat. SNc: substantia nigra pars compacta, VTA: ventral tegmental area.
6.3 Results

6.3.1 Experiment 1: Inhibition of the nigrostriatal pathway with DREADDs

*Effect of chronic CNO on choice behaviour.* In this group of rats, 35 rats were designated as wager-insensitive (n = 19 TH:cre positive, n = 16 TH:cre negative) and chose between the safe and uncertain lever fairly equally, regardless of the bet size in play (bet size: $F_{2,68} = 0.76, p = 0.471, \text{NS}$), whereas 28 (n = 12 TH:cre positive, n = 16 TH:cre negative) were designated as wager-sensitive due to their decrease in choice of the uncertain lever as the bet size increased (bet size: $F_{2,54} = 194.99, p < 0.001$). Chronic CNO did not affect choice behaviour during any of the 2 weeks of administration, regardless of baseline preference (Figure 6.2 A-B; Table 6.1; TH:cre group: all Fs < 0.25, all $p$s > 0.620, NS; Figure 6.2 D-E,G-H; TH:cre x wager-sensitivity: all Fs < 0.74, all $p$s > 0.392, NS; TH:cre x bet size: all Fs < 1.68, all $p$s > 0.191, NS). This lack of effect of inhibition of the nigrostriatal pathway is reminiscent to the lack of effect observed following bilateral dorsostriatal 6-OHDA lesions in Chapter 3.
Figure 6.2 Choice behaviour on the rBT during SNc inhibition and ropinirole

Figure 6.2. As per previous studies using this task, chronic ropinirole increased choice of the uncertain lever on the rBT, regardless of baseline preference. Data is shown for TH:cre negative and TH:cre positive rats. Percent choice of the uncertain lever pre-drug treatment (A), during inhibition of the SNc with CNO (B), and during CNO/ropinirole (C). The effect of ropinirole was not mediated by inhibition of the nigrostriatal pathway. Percent choice of the uncertain lever in the wager-sensitive (D-F) and wager-insensitive (G-I) rats. Choice behaviour in the non-responder (J) and responder rats (K). There was no true non-responder in this group of rats. Data shown are mean ± SEM
Table 6.1  Choice behaviour on the rBT during SNc inhibition and ropinirole

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<th>CNO</th>
<th>CNO/Ropinirole</th>
<th>Time point</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
<td>Week 3</td>
<td>Week 4</td>
</tr>
<tr>
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<td>45.99 ±</td>
<td>54.14 ±</td>
<td>52.33 ±</td>
<td>50.59 ±</td>
<td>47.15 ±</td>
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<tr>
<td></td>
<td></td>
<td>12.88</td>
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<td>Week 2</td>
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<td>55.72 ± 7.84</td>
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<td>48.20 ± 10.86</td>
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Non-choice variables. TH:cre positive rats made slightly less choice omissions at bet size 1 during the first week of CNO administration, and regardless of wager-sensitivity, an effect that disappeared by the second week of CNO administration (week 1- TH:cre group: $F_{1,58} = 6.82$, $p = 0.011$; TH:cre x bet size: $F_{2,116} = 3.31$, $p = 0.040$; TH:cre x sensitivity: $F_{1,58} = 0.08$, $p = 0.775$, NS; bet size 1: $F_{1,59} = 8.39$, $p = 0.005$; bet size 2: $F_{1,59} = 1.764$, $p = 0.189$, NS; bet size 3: $F_{1,59} = 0.92$, $p = 0.343$, NS; week 2- TH:cre group: $F_{1,58} = 1.86$, $p = 0.177$, NS; TH:cre x bet size: $F_{2,118} = 0.05$, $p = 0.948$, NS; TH:cre x sensitivity: $F_{1,58} = 0.00$, $p = 0.955$, NS; baseline: TH:cre group: $F_{1,58} = 0.45$, $p = 0.504$, NS; TH:cre x bet size: $F_{2,118} = 0.06$, $p = 0.941$, NS; TH:cre x sensitivity: $F_{1,58} = 0.00$, $p = 0.955$, NS). All other parameters were unaffected (choice and collection latencies, hole omission: all Fs < 3.584, all ps > 0.064, NS). Data values for all non-choice variables are given in Table 6.2.
Table 6.2  Other measurements on the rBT during SNc inhibiton with CNO

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<th>TH:Cre+</th>
<th>TH:Cre-</th>
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<td>Wager-Insensitive</td>
</tr>
<tr>
<td></td>
<td>Choice Latency-Safe</td>
<td>Choice Latency-Uncertain</td>
</tr>
<tr>
<td>1</td>
<td>2.12 ± 0.15</td>
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</tr>
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<tr>
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<td>1.68 ± 0.07</td>
<td>1.69 ± 0.06</td>
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<td></td>
<td>TH:Cre+</td>
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</tr>
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<td>2.01 ± 0.10</td>
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<td></td>
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<td>Choice Latency-Uncertain</td>
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</tbody>
</table>
**Effect of chronic ropinirole.** From the first week of training following osmotic pump surgery, chronic ropinirole increased choice of the uncertain lever across all bet sizes, regardless of animals’ baseline preference for the safe or uncertain option. Transgene status, and therefore down-regulation of dopamine release from the SNC, did not affect this response to ropinirole at any time point (Figure 6.2 C; Table 6.1; week 1- ropinirole treatment group: $F_{1,55} = 5.38, p = 0.024$; week 2- ropinirole treatment group: $F_{1,55} = 12.57, p = 0.001$; week 3- ropinirole treatment group: $F_{1,55} = 17.56, p = 0.001$; week 4- ropinirole treatment group: $F_{1,55} = 25.57, p < 0.001$; ropinirole treatment group x TH:cre: all Fs < 0.11, all ps > 0.741, NS; Figure 6.2 F,I; ropinirole treatment group x wager-sensitivity: all Fs < 0.48, all ps > 0.490, NS; ropinirole treatment group x TH:cre group x wager-sensitivity: all Fs < 0.86, all ps > 0.359, NS). Also, ropinirole did not differently affect the TH:cre positive and negative rats (ropinirole treated rats only- TH:cre group: all Fs < 1.37, all ps > 0.255, NS). We observed an interaction of ropinirole with bet size from the second week of ropinirole treatment. Although when looking at specific bet sizes, all comparisons between the saline and ropinirole group from the second week onward were significant, except for the smallest bet size during week 1 (week 1- bet size 1: $F_{1,55} = 3.90, p = 0.053$, NS; bet size 2: $F_{1,55} = 5.69, p = 0.021$; bet size 3: $F_{1,55} = 4.82, p = 0.032$; week 2-4, bet size 1-3: all Fs > 7.30, all ps < 0.009). The effect of ropinirole in increasing choice of uncertainty was also evident when each drug treatment group was compared to its respective baseline prior to surgery (Figure 6.2; ropinirole treatment week 4 vs baseline: ropinirole treatment group x time point: $F_{1,55} = 26.82, p < 0.001$; TH:cre group x time point, ropinirole treatment group x TH:cre group x time point: all Fs < 0.04, all ps > 0.457, NS; ropinirole-treated: $F_{1,36} = 64.19, p < 0.001$; saline-treated: $F_{1,19} = 0.58, p = 0.454$, NS).
In terms of individual differences in the response to ropinirole, analyses identified 31 out of 40 rats as truly “responders” (TH:cre positive: n = 14; TH:cre negative: n = 17; wager-sensitive: n = 15; wager-insensitive: n = 16) in that they showed a significant increase in preference for uncertainty in response to ropinirole when individually compared to their baseline. However, although rats in the non-responder group individually did not differ to their baseline, as a group, they showed an increase in choice of uncertainty and therefore cannot be considered a true “non-responder” group (Figure 6.2 J-K; ropinirole treatment week 4 vs baseline, responders vs non-responders: time point x group: $F_{1,32} = 9.48, p = 0.004$; responders only, time point: $F_{1,27} = 97.70, p < 0.001$; non-responders only; time point: $F_{1,5} = 20.66, p = 0.006$; analysis by TH:cre group; all Fs < 0.02, all $ps > 0.901$, NS; analysis by wager-sensitivity; all Fs < 1.13, all $ps > 0.297$, NS; number of responders/non-responders and TH:cre positive/TH:cre negative: $\chi^2(1) = 1.31, p = 0.451$, NS; number of responders/non-responders and wager-sensitive/wager-insensitive: $\chi^2(1) = 0.65, p = 0.476$, NS).

**Osmotic pump verification.** In order to confirm the proper functioning of the osmotic pumps, the level of ropinirole and saline solution remaining in the pumps at the time of euthanizing the rats following 28 days of chronic ropinirole was assessed. The start filling average for the solution was 1.97 ml ± 0.02 ml per osmotic pump. On average, 0.14 ml ± 0.01 ml was left in the pumps, with filling average of 1.97 ml at the start of the experiment, and no pump had more than 0.25 ml left at the end of the experiment. Given that the specific pump lot in this group of rats was delivering the drug for an average of 32.9 days, the amount of solution remaining after 28 days was not unexpected.

**Non-choice variables.** Data values for non-choice variables measured are given in Table 6.3. Consistent with the effect of chronic ropinirole observed in previous experiments in
chapters 3 and 5, rats receiving ropinirole made less hole omissions when compared to rats receiving saline. This effect began during the second week of drug administration and remained for the duration of chronic ropinirole (ropinirole treatment group- drug treatment week 1: $F_{1,52} = 0.17, p = 0.681, \text{NS}$; week 2: $F_{1,54} = 15.52, p < 0.001$; week 3: $F_{1,55} = 26.21, p < 0.001$; week 4: $F_{1,54} = 34.79, p < 0.001$). Nonetheless, during week 3 of ropinirole treatment, this effect was moderated by whether rats were wager-sensitive or wager-insensitive such that the wager-sensitive rats were more strongly affected by chronic ropinirole (week 3 & 4- ropinirole treatment x TH:cre group x sensitivity: all $Fs > 5.66$, all $ps < 0.021$; TH:cre positive/wager-sensitive only: ropinirole treatment: all $Fs > 27.97$, all $ps < 0.001$; wager-insensitive only: ropinirole treatment x TH:cre group: all $Fs < 1.10$, all $ps > 0.302$, NS; week 1 & 2- ropinirole treatment x TH:cre group x sensitivity: all $Fs < 3.74$, all $ps > 0.058$, NS). Chronic ropinirole also appeared to slightly decrease choice latency of the safe lever at bet size 1 during week 3 and 4 of treatment, although follow-up analyses could not detect a significant main effect at any one bet size (choice latency safe- week 1 & 2: all $Fs < 2.31$, all $ps > 0.125$, NS; week 3- ropinirole treatment x bet size: $F_{2,70} = 9.95, p < 0.001$; week 4- ropinirole treatment x bet size: $F_{2,72} = 3.24$, $p = 0.045$; week 3 & 4- bet size 1-3- ropinirole group: all $Fs < 3.34$, all $ps > 0.074$, NS).

Similarly, it seemed that rats receiving ropinirole were slightly slower at collecting reward compared to saline at bet size 2 after a safe lever during week 3, and also that ropinirole treated rats were faster at collecting reward at bet size 1 following choice of the risky lever during week 2 and 3, but these effect were very small and none of these were strong enough to show a significant difference between the ropinirole and saline groups at different bet sizes, suggesting that these effects were small and probably not due to a strong effect of the drug on behaviour (collection latency safe- week 3- ropinirole treatment x bet size: $F_{2,70} = 3.74, p = 0.029$; bet size
1-3- ropinirole group: all Fs < 3.50, all ps > 0.067, NS; all other weeks- ropinirole treatment x bet size: all Fs < 2.12, all ps > 0.128, NS; collection latency risky- week 2- ropinirole treatment x bet size: $F_{2,72} = 3.50$, $p = 0.036$; bet size 1-3- ropinirole group: all Fs < 1.41, all ps > 0.242, NS; week 3- ropinirole treatment x bet size: $F_{2,80} = 4.72$, $p = 0.012$; bet size 1-3- ropinirole group: all Fs < 1.26, all ps > 0.266, NS; all other weeks- ropinirole treatment x bet size: all Fs < 2.23, all ps > 0.302, NS).
Table 6.3 Other measurements on the rBT during SNc inhibiton and ropinirole

<table>
<thead>
<tr>
<th>Bet Size</th>
<th>Choice Latency-Safe</th>
<th>Choice Latency-Uncertain</th>
<th>Collection Latency-Safe</th>
<th>Collection Latency-Uncertain</th>
<th>Choice Omission</th>
<th>Hole Omission</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH:Cre+ Saline</td>
<td>1.93 ± 0.15</td>
<td>2.33 ± 0.35</td>
<td>0.42 ± 0.03</td>
<td>0.48 ± 0.04</td>
<td>0.06 ± 0.04</td>
<td>3.28 ± 0.61</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
<td>1.79 ± 0.16</td>
<td>1.78 ± 0.11</td>
<td>0.38 ± 0.02</td>
<td>0.47 ± 0.05</td>
<td>0.00 ± 0.05</td>
<td>2.40 ± 0.64</td>
</tr>
<tr>
<td>TH:Cre+ Saline</td>
<td>1.56 ± 0.11</td>
<td>1.73 ± 0.16</td>
<td>0.37 ± 0.03</td>
<td>0.42 ± 0.05</td>
<td>0.00 ± 0.05</td>
<td>3.51 ± 0.61</td>
</tr>
<tr>
<td>2</td>
<td>2.07 ± 0.15</td>
<td>1.80 ± 0.19</td>
<td>0.66 ± 0.23</td>
<td>0.65 ± 0.09</td>
<td>0.05 ± 0.05</td>
<td>4.50 ± 0.58</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
<td>1.87 ± 0.16</td>
<td>1.78 ± 0.17</td>
<td>0.43 ± 0.03</td>
<td>0.59 ± 0.09</td>
<td>0.00 ± 0.05</td>
<td>3.50 ± 0.79</td>
</tr>
<tr>
<td>TH:Cre+ Saline</td>
<td>1.66 ± 0.13</td>
<td>1.58 ± 0.13</td>
<td>0.43 ± 0.02</td>
<td>0.42 ± 0.03</td>
<td>0.90 ± 0.02</td>
<td>1.00 ± 0.56</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
<td>1.80 ± 0.10</td>
<td>1.53 ± 0.11</td>
<td>0.47 ± 0.06</td>
<td>0.40 ± 0.02</td>
<td>0.02 ± 0.02</td>
<td>0.85 ± 0.37</td>
</tr>
<tr>
<td>TH:Cre+ Ropinirole</td>
<td>1.66 ± 0.11</td>
<td>1.58 ± 0.13</td>
<td>0.43 ± 0.02</td>
<td>0.42 ± 0.03</td>
<td>0.03 ± 0.02</td>
<td>1.53 ± 0.60</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
<td>1.70 ± 0.13</td>
<td>1.89 ± 0.37</td>
<td>0.43 ± 0.04</td>
<td>0.59 ± 0.10</td>
<td>0.05 ± 0.05</td>
<td>3.80 ± 1.04</td>
</tr>
<tr>
<td>TH:Cre+ Ropinirole</td>
<td>1.71 ± 0.09</td>
<td>1.53 ± 0.12</td>
<td>0.46 ± 0.05</td>
<td>0.90 ± 0.43</td>
<td>0.03 ± 0.03</td>
<td>1.00 ± 0.40</td>
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<tr>
<td>Wager-Sensitive</td>
<td>1.63 ± 0.06</td>
<td>1.61 ± 0.14</td>
<td>0.44 ± 0.04</td>
<td>0.48 ± 0.07</td>
<td>0.03 ± 0.03</td>
<td>0.35 ± 0.12</td>
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<tr>
<td>TH:Cre+ Saline</td>
<td>1.55 ± 0.04</td>
<td>1.65 ± 0.14</td>
<td>0.43 ± 0.03</td>
<td>0.46 ± 0.06</td>
<td>0.00 ± 0.00</td>
<td>1.91 ± 0.60</td>
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<td>Wager-Insensitive</td>
<td>1.89 ± 0.18</td>
<td>1.47 ± 0.09</td>
<td>0.39 ± 0.02</td>
<td>0.38 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>2.27 ± 0.60</td>
</tr>
<tr>
<td>TH:Cre+ Saline</td>
<td>2.03 ± 0.22</td>
<td>1.91 ± 0.23</td>
<td>0.38 ± 0.03</td>
<td>0.41 ± 0.03</td>
<td>0.03 ± 0.03</td>
<td>3.93 ± 0.64</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
<td>1.79 ± 0.20</td>
<td>1.80 ± 0.24</td>
<td>0.35 ± 0.03</td>
<td>0.37 ± 0.02</td>
<td>0.03 ± 0.03</td>
<td>2.70 ± 0.58</td>
</tr>
<tr>
<td>TH:Cre+ Saline</td>
<td>1.74 ± 0.21</td>
<td>1.65 ± 0.20</td>
<td>0.35 ± 0.02</td>
<td>0.35 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>4.87 ± 0.68</td>
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<tr>
<td>Wager-Insensitive</td>
<td>1.84 ± 0.13</td>
<td>1.59 ± 0.09</td>
<td>0.44 ± 0.03</td>
<td>0.51 ± 0.04</td>
<td>0.03 ± 0.03</td>
<td>2.80 ± 0.89</td>
</tr>
<tr>
<td>TH:Cre+ Saline</td>
<td>1.76 ± 0.09</td>
<td>1.53 ± 0.07</td>
<td>0.41 ± 0.02</td>
<td>0.48 ± 0.04</td>
<td>0.00 ± 0.00</td>
<td>1.40 ± 0.44</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
<td>1.58 ± 0.08</td>
<td>1.66 ± 0.12</td>
<td>0.40 ± 0.01</td>
<td>0.44 ± 0.04</td>
<td>0.00 ± 0.00</td>
<td>2.57 ± 0.49</td>
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<tr>
<td>TH:Cre+ Ropinirole</td>
<td>1.72 ± 0.15</td>
<td>1.66 ± 0.08</td>
<td>0.41 ± 0.03</td>
<td>0.42 ± 0.03</td>
<td>0.04 ± 0.03</td>
<td>1.30 ± 0.46</td>
</tr>
<tr>
<td>Wager-Insensitive</td>
<td>1.75 ± 0.07</td>
<td>1.76 ± 0.08</td>
<td>0.39 ± 0.02</td>
<td>0.43 ± 0.03</td>
<td>0.02 ± 0.02</td>
<td>1.08 ± 0.64</td>
</tr>
<tr>
<td>TH:Cre+ Ropinirole</td>
<td>1.69 ± 0.07</td>
<td>1.63 ± 0.07</td>
<td>0.39 ± 0.02</td>
<td>0.41 ± 0.03</td>
<td>0.00 ± 0.00</td>
<td>2.22 ± 0.60</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
<td>1.81 ± 0.18</td>
<td>1.52 ± 0.13</td>
<td>0.41 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.02 ± 0.02</td>
<td>0.85 ± 0.26</td>
</tr>
<tr>
<td>2</td>
<td>2.11 ± 0.19</td>
<td>1.59 ± 0.09</td>
<td>0.39 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.06 ± 0.04</td>
<td>0.92 ± 0.38</td>
</tr>
<tr>
<td>Wager-Sensitive</td>
<td>1.71 ± 0.11</td>
<td>1.54 ± 0.09</td>
<td>0.37 ± 0.02</td>
<td>0.39 ± 0.03</td>
<td>0.04 ± 0.04</td>
<td>3.25 ± 0.95</td>
</tr>
</tbody>
</table>
Rats in the ropinirole treated group were also faster to choose the risky lever at bet size 1 during the last two weeks of treatment (week 1-4: ropinirole treatment x bet size: all Fs > 5.70, all ps < 0.045; week 1 & 2- bet size 1-3- ropinirole group: all Fs < 2.77, all ps > 0.104, NS; week 3- bet size 1- ropinirole group: $F_{1,60} = 6.31, p = 0.015$; week 4- bet size 1- ropinirole group: $F_{1,59} = 6.02, p = 0.017$; bet size 2 & 3- ropinirole group: all Fs < 0.80, all ps > 0.375, NS). Ropinirole did not affect any other variables, regardless of TH:cre group or wager-sensitivity (ropinirole treatment, ropinirole treatment x TH:cre group, ropinirole treatment x wager-sensitivity, ropinirole treatment x TH:cre group x wager-sensitivity: all Fs < 2.59, all ps > 0.117, NS).

*Forelimb adjusting step test.* TH:cre positive rats made fewer forelimb adjusting steps 1.5 week following the start of chronic CNO compared to TH:cre negative rats (Figure 6.3; TH:cre group: $t_{61} = 2.19, p = 0.033$). However, this difference was no longer evident 3 weeks following the start of chronic ropinirole, regardless of whether rats were in the saline or ropinirole treated group, indicating that mechanisms other than the effect of ropinirole on motor behaviour accounted for this reduced difference between the groups (TH:cre group: $t_{61} = 0.82, p = 0.414$, NS; ropinirole treatment group: $t_{61} = 0.19, p = 0.850$, NS; TH:cre positive only-ropinirole vs saline: $t_{29} = 0.79, p = 0.434$, NS).
Figure 6.3 Assessment of motor behaviour on the forelimb adjusting step test

Figure 6.3. Number of adjusting steps 1.5 weeks following start of CNO (A), and 3.5 weeks following initiation of chronic ropinirole (B). CNO slightly decreased adjustment steps in TH:cre positive rats. However, this difference was no longer evident during ropinirole treatment. Data shown are mean ± SEM.
6.3.2  Experiment 2: Excitation of the nigrostriatal pathway with DREADDs

Choice behaviour. 19 rats (n = 11 TH:cre positive, n = 8 TH:cre negative), were
designated as wager-insensitive due to their relatively equal sampling between the safe and
uncertain lever, regardless of the bet size in play (bet size: $F_{2,36} = 0.85, p = 0.438, \text{NS}$), whereas
13 rats (n = 5 TH:cre positive, n = 8 TH:cre negative) were designated as wager-sensitive due to
their decrease in choice of the uncertain lever as the bet size increased (bet size: $F_{2,24} = 81.05, p$
< 0.001).

Effect of chronic CNO. Chronic excitation of the nigrostriatal pathway with CNO
partially replicated the effect of chronic ropinirole observed previously. During week 2 of CNO
administration, visual inspection of the data suggested that TH:cre positive wager-sensitive rats
increased their choice of the uncertain lever. This increase in preference for uncertainty became
significant at the end of week 4 of drug treatment (Figure 6.4 A-B; Table 6.4; week 4- TH:cre
group: $F_{1,28} = 0.08, p = 0.780, \text{NS}$; Figure 6.4 C-F; TH:cre x wager-sensitivity: $F_{1,28} = 4.51, p =$
0.043; TH:cre x bet size: $F_{2,56} = 1.01, p = 0.369, \text{NS}$; TH:cre x wager-sensitivity x bet size: $F_{2,56}$
= 1.65, $p = 0.201, \text{NS}$; all other weeks: TH:cre group: all Fs < 3.38, all $ps > 0.077, \text{NS}$; TH:cre x
wager-sensitivity: all Fs < 3.58, all $ps > 0.069, \text{NS}$; TH:cre x bet size: all Fs < 1.38, all $ps >$
0.259, NS; TH:cre x wager-sensitivity x bet size: all Fs < 1.67, all $ps > 0.198, \text{NS}$). The effect of
excitation of the nigrostriatal pathway with CNO was also evident when rats were compared to
their respective baseline, however further analysis within each wager-sensitive/wager-insensitive
group could only detect trend level change from baseline (Figure 6.4 C-F; baseline vs week 4-
time point x TH:cre group x wager-sensitivity: $F_{1,28} = 4.49, p = 0.043$; wager-sensitive only- time
point x TH:cre: $F_{1,11} = 4.16, p = 0.066, \text{NS}$; wager-insensitive only- time point: $F_{1,17} = 0.25, p =$
0.627, NS). We did not attempt to identify other responder rats since the effect of chronic CNO was evidently driven by the wager-sensitive rats (n = 5).
Figure 6.4 Choice behaviour on the rBT during SNC excitation

Figure 6.4. Chronic inhibition of SNC increased choice of uncertainty in wager-sensitive female rats. Data is shown pre-drug treatment with CNO and during chronic CNO. Percent choice of the uncertain lever in TH:cre negative (A) and TH:cre positive (B) rats. Percent choice of the uncertain lever in the wager-sensitive (C-D) and wager-insensitive (E-F) rats. Data shown are mean ± SEM.
### Table 6.4 Choice behaviour on the rBT during SNC excitation

<table>
<thead>
<tr>
<th>TH:Cre+</th>
<th>Time point</th>
<th>Bet Size</th>
<th>Pre drug-treatment</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wager-Insensitive</td>
<td>1</td>
<td></td>
<td>52.78 ± 7.93</td>
<td>38.58 ± 5.55</td>
<td>48.66 ± 6.14</td>
<td>53.18 ± 5.08</td>
<td>49.80 ± 4.62</td>
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<tr>
<td></td>
<td>2</td>
<td></td>
<td>52.74 ± 8.54</td>
<td>38.99 ± 6.12</td>
<td>43.43 ± 6.88</td>
<td>52.41 ± 6.30</td>
<td>49.49 ± 6.39</td>
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<tr>
<td></td>
<td>3</td>
<td></td>
<td>48.46 ± 9.27</td>
<td>38.52 ± 7.29</td>
<td>47.05 ± 7.88</td>
<td>48.92 ± 7.72</td>
<td>45.72 ± 8.31</td>
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<td>Wager-Sensitive</td>
<td>1</td>
<td></td>
<td>55.57 ± 3.32</td>
<td>55.90 ± 9.95</td>
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<td></td>
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<td></td>
<td>34.68 ± 5.08</td>
<td>38.00 ± 6.51</td>
<td>50.61 ± 9.92</td>
<td>55.28 ± 11.74</td>
<td>52.60 ± 12.25</td>
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<tr>
<td></td>
<td>3</td>
<td></td>
<td>16.82 ± 1.82</td>
<td>23.81 ± 3.04</td>
<td>35.62 ± 9.17</td>
<td>42.54 ± 14.46</td>
<td>44.27 ± 12.98</td>
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<td>TH:Cre-</td>
<td>1</td>
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<td>63.76 ± 3.69</td>
<td>67.39 ± 5.66</td>
<td>63.71 ± 7.02</td>
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<td>63.97 ± 8.78</td>
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<td>59.27 ± 6.60</td>
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</tbody>
</table>
**Non-choice variables.** Data values for non-choice variables measured are given in Table 6.5. TH:cre positive rats made slightly less hole omissions at bet size 1 regardless of wager-sensitivity, but only during the second week of chronic CNO (week 2- TH:cre group: $F_{1,27} = 0.05, p = 0.833$, NS; TH:cre x bet size: $F_{2,56} = 3.23, p = 0.047$; TH:cre x sensitivity: $F_{1,27} = 0.42, p = 0.522$, NS; bet size 1- TH:cre group: $F_{1,30} = 6.08, p = 0.020$; bet size 2- TH:cre group: $F_{1,30} = 0.06, p = 0.817$, NS; bet size 3- TH:cre group: $F_{1,30} = 1.44, p = 0.240$, NS; all other weeks-TH:cre group: all Fs < 0.41, all $ps > 0.525$, NS; TH:cre x bet size: all Fs < 0.93, all $ps > 0.397$, NS; TH:cre x sensitivity: all Fs < 0.79, all $ps > 0.381$, NS). We also found an interaction effect such that TH:cre positive rats appeared faster at choosing the safe lever at bet size 1 during week 2 and at both bet size 1 and 2 during week 4, but this effect was really small and further analyses did not reveal a difference between the group at any of the bet size (week 2- TH:cre x bet size: $F_{2,42} = 3.56, p = 0.037$; bet size 1-3- TH:cre group: all Fs < 1.29, all $ps > 0.267$, NS; week 4-TH:cre x bet size: $F_{2,42} = 3.70, p = 0.033$; bet size 1-3- TH:cre group: all Fs < 2.50, all $ps > 0.127$, NS). Also, it looked like the TH:cre positive wager sensitive rats were faster at collecting reward after choice of a safe lever at bet size 1, but once again, further analysis at each bet size did not reveal a significant difference between the TH:cre positive and negative rats at any of the bet size. This difference was also very small in magnitude and did not appear to be of behavioural significance (week 4- TH:cre x bet size x wager-sensitivity: $F_{2,42} = 3.37, p = 0.044$; TH:cre positive only: bet size x wager-sensitivity: $F_{2,22} = 8.35, p = 0.002$; wager-sensitive only: bet size 1-3- TH:cre group: all Fs < 0.27, all $ps > 0.618$, NS; TH:cre negative only: bet size x wager-sensitivity: $F_{2,20} = 0.50, p = 0.617$, NS). All other parameters were unaffected (choice and collection latency risky, choice omission: all Fs < 3.639, all $ps > 0.071$, NS).
### Table 6.5 Other measurements on the rBT during SNc excitation

<table>
<thead>
<tr>
<th>Bet Size</th>
<th>Choice Latency Safe</th>
<th>Choice Latency Uncertain</th>
<th>Collection Latency Safe</th>
<th>Collection Latency Uncertain</th>
<th>Choice Omission</th>
<th>Hole Omission</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH:Cre+ Wager-Insensitive</td>
<td>1</td>
<td>1.81 ± 0.13</td>
<td>2.00 ± 0.25</td>
<td>0.45 ± 0.02</td>
<td>0.46 ± 0.04</td>
<td>0.09 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.86 ± 0.09</td>
<td>1.71 ± 0.12</td>
<td>0.42 ± 0.02</td>
<td>0.47 ± 0.04</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.72 ± 0.09</td>
<td>1.61 ± 0.09</td>
<td>0.40 ± 0.02</td>
<td>0.45 ± 0.04</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>TH:Cre+ Wager-Sensitive</td>
<td>1</td>
<td>1.82 ± 0.14</td>
<td>1.43 ± 0.10</td>
<td>0.43 ± 0.03</td>
<td>0.50 ± 0.09</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.70 ± 0.09</td>
<td>1.49 ± 0.09</td>
<td>0.41 ± 0.03</td>
<td>0.48 ± 0.09</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.59 ± 0.12</td>
<td>1.50 ± 0.15</td>
<td>0.42 ± 0.05</td>
<td>0.48 ± 0.09</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>TH:Cre+ Wager-Insensitive</td>
<td>1</td>
<td>2.11 ± 0.18</td>
<td>1.95 ± 0.15</td>
<td>0.45 ± 0.03</td>
<td>0.53 ± 0.08</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.16 ± 0.14</td>
<td>1.83 ± 0.11</td>
<td>0.42 ± 0.02</td>
<td>0.52 ± 0.08</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.81 ± 0.06</td>
<td>1.73 ± 0.10</td>
<td>0.40 ± 0.02</td>
<td>0.50 ± 0.07</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>TH:Cre- Wager-Sensitive</td>
<td>1</td>
<td>1.96 ± 0.17</td>
<td>1.85 ± 0.18</td>
<td>0.47 ± 0.04</td>
<td>0.51 ± 0.04</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.90 ± 0.14</td>
<td>1.79 ± 0.14</td>
<td>0.43 ± 0.02</td>
<td>0.48 ± 0.04</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.74 ± 0.13</td>
<td>1.88 ± 0.12</td>
<td>0.43 ± 0.02</td>
<td>0.44 ± 0.05</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>
6.4 Discussion

In the first experiment in this chapter, we unequivocally showed that ropinirole has a strong and reliable effect on gambling-like decision-making by replicating once again that chronic ropinirole leads to a dramatic increase in choice of uncertainty in male rats performing on the rBT. We also showed that, although the effect of ropinirole was manifested slightly sooner as compared to previous studies, inhibition of the nigrostriatal pathway with DREADDs did not strongly affect the response to ropinirole. Nonetheless, inhibition of the nigrostriatal pathway led to a slight decrease in forepaw adjusting steps, an effect which was no longer evident three weeks later, regardless of whether rats received ropinirole or saline. As in Chapter 5, we were unable to differentiate a truly non-responder group, with most rats being affected by ropinirole. Ropinirole also appeared to affect parameters of decision-making such as reducing choice latency of the risky lever and hole omission, similar to previous studies.

The most exciting finding in our group of female rats performing the rBT was the dramatic increase in choice of uncertainty in TH:cre positive wager-sensitive rats following chronic excitation of the nigrostriatal pathway during chronic CNO administration. This effect somewhat mimics the effect observed with chronic ropinirole in Chapters 3 and 5 (Tremblay et al, 2016). Also similar to the effect of ropinirole treatment, CNO decreased hole omissions in TH:cre positive rats.

The results from experiment 1 suggests once again that damage to the nigrostriatal system may not be necessary for the development of ICDs in PD patients, but that ropinirole treatment alone can make patients vulnerable to these side-effect of the drug. If damage to the nigrostriatal dopamine system was in play such as suggested in the overdose hypothesis (Cools, 2006; Kish et al, 1988), we would expect that TH:cre positive rats would show a stronger
increase in choice of the uncertain option during ropinirole treatment as compared to TH:cre negative rats, which was not the case here. However, some explanations may have contributed to this lack of differences between the TH:cre positive and negative rats in response to ropinirole. CNO can be metabolized into clozapine and N-desmethylclozapine in rodents, humans and non-human primates (Chang et al, 1998; MacLaren et al, 2016; Raper et al, 2017). Clozapine could have affected both the TH:cre positive and negative rats, dampening our ability to see a difference between these two groups in response to ropinirole. Clozapine acts as a D₂ receptor antagonist, as well as a 5-HT₂A/2C antagonist, and has shown some efficacy against tremor in PD, (Bonuccelli et al, 1997; Carlson et al, 2003), and in increasing the “on” time in L-DOPA treated patients (Durif et al, 2004), suggesting an interaction of clozapine with dopaminergic drugs. However, doses of 1 mg/kg of CNO have been widely used in behavioural studies in rodents, with minimal effects in animals not expressing DREADDs (i.e. Smith et al, 2016; Urban et al, 2015; Whissell et al, 2016 for review). The effect of clozapine on performance of the rBT is unknown, but the fact that CNO only affected TH:cre positive rats in the second experiment suggests that the reverse metabolism of CNO into clozapine does not cause any behavioural change on its own. Furthermore, even if clozapine was acting to suppress dopaminergic transmission in all rats, we would expect an additional effect of CNO in transgene positive animals due to the expression of the DREADD. However, this was clearly absent.

With regards to the null effects in experiment 1, it could also be argued that we failed to reliably down-regulate dopamine release because CNO was delivered in a pulsatile fashion, rather than chronically. CNO levels peak within 30 minutes, after which CNO levels decline over the following 2 hours after systemic administration (Guettier et al, 2009). However, behavioural effects can be observed for up to 6 hours following administration, suggesting a long
lasting effect of CNO. We also injected CNO 30 minutes prior to testing on the rBT or performing the forelimb adjusting step test to maximize the chances of observing a behavioural effect (Alexander et al., 2009). Nonetheless, future studies should try delivering CNO in an osmotic pump in order to assess its effect in a more continuous and stable release.

The loss of dopamine neurons in the substantia nigra in PD leads to marked motor deficits (Wilson et al., 1996). Similarly, we hypothesized that inhibition of dopaminergic input from the substantia nigra to the dorsal striatum in rats would lead to motor deficits on the forelimb adjusting step test, similar to the effect of other animal models such as the bilateral dorsolateral 6-OHDA lesion model (Olsson et al., 1995). We observed a reduction in adjustment steps in the TH:cre positive rats 1.5 weeks following the start of chronic CNO, suggesting that DREADDs inhibition had an effect on motor activity similar to other models of PD which showed deficits on the step test. However, there was no such difference between the TH:cre positive and negative rats 3 weeks following implantation of the osmotic pump, regardless of whether rats were receiving ropinirole or saline, suggesting that the deficit may have been overcome by unknown compensatory mechanisms. Since DREADDs receptors are GPCRs, they can show the same characteristics as the endogenous receptors. Therefore, when chronically activated such as in our study, DREADDs receptors can desensitize and downregulate, similar to chronic activation of GPCRs (DeWire et al., 2007). It is believed that desensitization depends on receptor availability, a term called “receptor reserve”, in which a maximum response to a drug can be achieved with lower concentration of that drug when a larger number of receptors are expressed on a cell (Ruffolo, 1982). In the study here, it is unknown whether chronic CNO led to desensitization and downregulation of receptors, which could explain the equalization of performance on the step test. Also, if desensitization occurred prior to ropinirole administration,
this could potentially also explain why we did not see a potentiation of the effect of chronic ropinirole on behaviour. However, previous studies have seen no desensitization effect in DREADDs receptors (Alexander et al., 2009; Krashes et al., 2011), which was explained by the fact that if cells express a high number of DREADDs receptors compared to endogenous receptors, the lower occupancy of these DREADDs receptors with the agonist will make the cell less sensitive to repeated administration. Therefore, this mechanism is complex and depends on an intricate ratio of DREADDs and endogenous receptor expression, along with doses and number of CNO administration (also see Roth, 2016). Further studies will be required in order to determine if such a mechanism was in play here.

Not all cells in a targeted area will express DREADDs receptors following AAV vector infusion (Gremel et al., 2013; Smith et al., 2016). About 66% of cells will express DREADDs receptors, and of those, it is believed that only about 80% would be inhibited with CNO administration, reaching about 60% of their baseline activation (Chang et al., 2015). Therefore, activity in a brain area is reduced with DREADDs rather than completely eliminated such as seen with microinfusions (Smith et al., 2016). In PD, close to 80% of dopamine neurons have died by the time symptoms of the disease appear (Wilson et al., 1996), which suggests a significant loss in dopamine modulation in this system that exceed what would be expected with DREADDs technology. However, the null effect of inhibition of the nigrostriatal pathway with DREADDs on the response to ropinirole matches the effect of DRTs in previous studies using lesion models (Rokosik et al., 2012; Tremblay et al., 2016), as well as by the development of DRT-ICDs in patients for which dopamine depletion is not responsible for the disease such as restless leg syndrome and fibromyalgia (Holman, 2009; Quickfall et al., 2007; Tippmann-Peikert et al., 2007).
In the second experiment, chronic excitation of the nigrostriatal pathway with CNO led to a dramatic increase in choice of uncertainty in female transgenic wager-sensitive rats performing the rBT, somewhat mimicking the effect of chronic ropinirole in our previous studies (see Chapters 3 and 5). In contrast to inhibition with DREADDs, excitation of hM3D receptor coupled to Gq signaling increases burst firing by activating the ERK1/2 pathway (Alexander et al., 2009) and appears to be more efficient compared to inhibition with DREADDs, with studies showing that most cells fire following systemic CNO administration, and that excitation was more than double that at baseline (Vazey et al., 2014a).

Although we had planned to do this experiment in male rats for consistency with our previous experiments, the restricted availability of male rats due to a 50% probability of male and female rats in a litter did not allow us to use all 95 male rats for these two experiments. Nonetheless, awareness of the importance to study the effect of sex differences in research is rapidly increasing. In the gambling literature, middle-aged women are disproportionally represented in the demographic of slot machine players (Blaszczynski and Nower, 2002; Petry, 2003). In PD, women appear to present with symptoms two years older than men, an effect potentially due to the effect of estrogens on DAT levels in women (Haaxma et al., 2007; Twelves et al., 2003). Although the risk for PD, as well as for developing ICDs in response to DRT is higher in men (i.e. Dagher et al., 2009), women can also develop PD. In addition, women also present in higher proportion than men with diseases such as fibromyalgia and restless leg syndrome in which dopamine depletion is not involved in the disease, but in which treatment with DRT can also lead to ICDs (Holman, 2009; Quickfall et al., 2007; Tippmann-Peikert et al., 2007). Given that both male and females can develop DRT-ICD, our use of female rats in this study is therefore valid.
It is also interesting that we saw an effect of chronic CNO that was driven by the wager-sensitive rats in this study. Although we observed a more important increase in preference for uncertainty in the wager-sensitive rats in experiment 1 in Chapter 3, the analysis by wager-sensitivity was not significant, indicating that the effect of ropinirole affected most of the rats, independently of baseline preference for the uncertain or safe option. Therefore, the differential effect seen here may be due to differences in monoamine transmission between wager-sensitive and wager-insensitive rats, or differences in the function of DREADDs vs ropinirole treatment. Wager-sensitivity on the rBT was associated with lower D_{2/3} receptor expression in the dorsal striatum in (male) rats (Cocker et al., 2012a). As noted earlier, a higher ratio of DREADDs to endogenous receptors would make the cell less sensitive to repeated administration of CNO and therefore reduce the desensitization and internalization of receptors as a compensatory mechanism. Although somewhat speculative, this may explain why wager-sensitive animals were more sensitive to CNO, as compared to wager-insensitive rats.

Inactivation of the lateral OFC increased preference for uncertainty only in wager-sensitive rats on this task (Barrus et al., 2016a), somewhat mimicking the effect of chronically exciting the nigrostriatal pathway with DREADDs in this study. Given the strong connections of the OFC with the dorsal striatum and its involvement in updating the subjective value of rewards, it is perhaps not surprising that activation of this pathway had the greatest effect in wager-sensitive rats as these animals are relying to a greater degree on subjective, rather than objective, evaluation of reward when making decisions on the rBT. Alternatively, excitation of the nigrostriatal pathway may have impaired learning from negative feedback, as outlined in the introduction, such that reward omission on the uncertain lever was less aversive. However, if so,
we would expect an increase in choice of the uncertain option in all rats, rather than the selective effect on wager-sensitive rats observed here.

The fact that we used female rats in the current study, and male rats in all our ropinirole challenge studies, prevents us from definitively concluding that the effects of ropinirole are qualitatively different from the effect of chronically activating the nigrostriatal pathway with DREADDs. However, the two manipulations clearly have different pharmacological mechanisms. The chemogenetics approach used here would result in increased dopamine release from neurons originating within the substantia nigra, which largely (though not exclusively, see Blandini et al, 2000; Haleem, 2015; Huot et al, 2011; Lanciego et al, 2012) project to the striatum, but would affect all dopaminergic receptors. In contrast, systemic administration of ropinirole selectively activates pre- and post-synaptic $D_2/3$ receptors, but throughout the brain. As such, the additional effects of ropinirole (i.e. the increase in preference for the uncertain outcome observed in wager-insensitive rats) may not be mediated within the dorsal striatum.

Rather than simply boosting choice of the uncertain outcome in wager-sensitive rats, an alternative interpretation is that increasing dopamine release in the nigrostriatal pathway ameliorated wager-sensitivity, such that animals no longer showed a change in preference for the uncertain outcome based on bet size. Previous data suggest that dissociable neurochemical mechanisms are involved in the manifestation of wager-sensitivity- i.e. the degree to which bet size modulates choice of the uncertain option, and in the general preference for uncertainty: amphetamine boosts the latter without affecting the former, whereas eticlopride selectively induces wager-sensitive choice in wager-insensitive rats (Cocker et al. 2012). Repeated amphetamine increases choice of uncertain outcomes in a probability discounting task (Floresco and Whelan, 2009). Given the role of the NAc in encoding animal’s preferred option, regardless
of whether it is the most valuable option (Sugam et al., 2012), it is therefore possible that ropinirole amplifies choice of the uncertain outcome in wager-insensitive rats through this region.

Finally, ropinirole and excitation of the nigrostriatal pathway with DREADDs had similar effects on reducing hole omissions, whereas only chronic ropinirole decreased choice latency. As suggested in previous chapters, decreased omissions may suggest an increased engagement with the task, which was somewhat mirrored in the increase in trials completed on the rodent slot machine task (Cocker et al., 2017), and similar to the compulsive engagement observed in gambling and other forms of addictions. Previous studies have also shown an increase in the rapidity of decision-making following dopaminergic drug treatment in PD patients with ICDs (Voon et al., 2010). At least some aspects of this putative compulsive-like task engagement may therefore be mediated through elevated dopamine release in the dorsal striatum.

In summary, these studies suggests that inhibition of the nigrostriatal pathway is not necessary for DRT-induced ICDs as suggested by the lack of effect of inhibition of this pathway with DREADDs. Also, it appears that excitation of the nigrostriatal pathway with DREADDs may be involved in the development of DRT-ICDs, but that the mechanisms underlying ropinirole- vs DREADDs-induced increase in preference for uncertainty may slightly differ as suggested by the stronger effect of DREADDs excitation on wager-sensitive rats.
Chapter 7: General discussion

7.1 Summary of experimental findings

Preferential dopamine D_{2/3} agonists such as ropinirole and pramipexole have been successfully developed and are highly effective for the motor symptoms of PD, either as alternative or adjunct therapeutics to L-DOPA. However, the use of these dopamine replacement therapies (DRT) can lead to devastating psychiatric complications such as gambling disorders (GD) and other impulse control disorders (ICDs) in a significant minority of patients (Voon et al., 2011b; Weintraub et al., 2010; Weintraub et al., 2006a; Weintraub et al., 2006b). Studies have also shown that DRT can precipitate ICDs in other patient groups for which they are prescribed, including restless leg syndrome and fibromyalgia (Holman, 2009; Quickfall et al., 2007; Voon et al., 2011c), reducing neurologists’ inclination to prescribe them (i.e. Zhang et al., 2016), and leaving patients with little options beside deep brain stimulation, which is highly invasive. Whether previous tendencies towards risk can predict the onset of DRT-ICDs, and the specific mechanisms by which these DRT-ICDs occur, are unknown. Therefore, our studies in Chapter 3 attempted to determine the contribution of dopamine loss as a result of PD and prior tendencies toward risk in the development of ICDs. Using the rat betting task (rBT), a task designed to capture the degree to which animals’ preference for a safe or uncertain option may change as a function of the bet size in play, we assessed the response to chronic ropinirole in otherwise-healthy rats and in a dorsolateral striatal 6-OHDA lesion model of PD (Baunez et al. 1995, 2007, Rokosik and Napier 2012).

We showed that chronic slow-release ropinirole treatment via osmotic mini-pump implanted subcutaneously dramatically increased preference for the uncertain option on the rBT.
We also demonstrated that the ability of ropinirole to bias choice towards the probabilistic delivery of rewards on this task was not altered by 6-OHDA lesions of the dorsolateral striatum. Furthermore, individual differences in preference for the safe or uncertain option at baseline did not predict the response to ropinirole treatment, but it became clear that a subgroup of responder rats drove this effect. Therefore, we suggest that the altered judgment exhibited by patients with PD or restless leg syndrome following chronic DRT treatment can be largely attributed to the direct actions of the drugs themselves (Rokosik et al., 2012; Weintraub, 2008), rather than resulting from an interaction between the medication and basal risk preference, or any change in dopamine function caused by the diseases for which they were originally prescribed. In all studies using ropinirole we also observed a decrease in omissions, potentially suggesting that ropinirole invigorates performance and leads to a stronger engagement with the task, similar to the effects of this drug treatment on a rat slot machine task (Cocker et al., 2017).

Decision-making deficits have been observed in a variety of tasks in patients with PD on DRTs, but the influence of differences in task demands and the specific neural network recruited in task performance in precipitating these deficits is unclear. Although GD individuals are impaired on the IGT, the literature on performance of PD patients with or without ICDs on this task is mixed (Bentivoglio et al., 2013; Euteneuer et al., 2009; Gescheidt et al., 2012; Kobayakawa et al., 2008; Mimura et al., 2006; Pagonabarraga et al., 2007; Pignatti et al., 2012; Rossi et al., 2010). In Chapter 4, we therefore tested whether animals performing the rGT, a task analogous to the IGT used clinically, would show deficits on this task during chronic ropinirole. We also examined whether the addition of cues would trigger an effect of ropinirole given the involvement of cues in GD and in substance addiction (Grant et al., 2015; Obrien et al., 1992; van Holst et al., 2012a).
We showed that chronic ropinirole had a destabilizing effect on choice behaviour in rats performing the cued rGT that was not specific to any of the various options chosen, and did not depend on whether animals were risk-preferring or optimal decision-makers at baseline, but we saw no such effect of ropinirole on choice on the uncued version of the task. This was in marked contrast to the strong effect of ropinirole in increasing choice of uncertainty in the rBT (Tremblay et al, 2016), supporting the hypothesis that performance on the rBT and rGT may be mediated by different brain areas (Cocker et al, 2015; Winstanley et al, 2016a). Nonetheless, chronic ropinirole increased the number of premature responses made, a measure of impulsivity, on both versions of the rGT, an effect that was significantly more pronounced and long lasting on the cued version of the task. Also consistent with observations on the rBT, we saw an increase in task engagement, again potentially reminiscent of increased motivation to gamble in GD.

Converging lines of evidence pointed to GSK3β as a mechanistic driver of the behavioural effects of ropinirole, given the involvement of this signaling protein in psychiatric disorders such as drug addiction, schizophrenia and bipolar disorder, as well as in hyperdopamine-dependant behaviours (Beaulieu et al, 2005; Beaulieu et al, 2004; Peterson et al, 2015b). In addition, valproate and lithium, both of which are potent inhibitors of GSK3β, have shown at least partial success in treating GD (Beaulieu et al, 2005; Hollander et al, 2005; Pallanti et al, 2002). In Chapter 5, we therefore subcutaneously implanted a new group of healthy rats performing the rBT with an osmotic pump delivering ropinirole or saline as per experiments in Chapter 3, and attempted to block or diminish the increase in uncertain choices induced by ropinirole with the GSK3β inhibitor SB 216763.
We replicated the behavioural effect of chronic ropinirole by showing that this drug leads to a strong and reliable increase in preference for uncertainty on the rBT. Interestingly, we were unable to block this effect with chronic SB 216763. Protein analysis using Western blot revealed that rats classified as responders in Chapter 3 showed a trend towards higher levels of GSK3β in the dorsal striatum, yet no change in CREB or pDARPP34, indicative of predominant activation of the D2-mediated β-arrestin signaling pathway. In contrast, ropinirole-treated animals that did not shift their choice preference in response to the drug showed lower levels of pDARPP34 and CREB in this area, yet no change in GSK3β, suggesting that D2-mediated PKA-dependent signaling prevailed. In the second experiment, ropinirole-treated rats showed a more global increase in levels of GSK3β regardless of whether rats received the inhibitor SB 216763, an effect that was not significant in the saline-treated animals. Also, in contrast to the previous study, animals receiving chronic ropinirole and vehicle showed a decrease in D2 receptors, an effect that may have been blocked by SB 216763.

The “overdose” hypothesis, in which DRT treatment would restore functioning in the dorsal striatum and improve movement in PD patients, but overwhelm the mostly spared mesolimbic reward system, was suggested to explain the development of ICDs in PD (Cools, 2006; Kish et al, 1988). This theory emphasizes the involvement of the ventral mesolimbic system in ICDs such as gambling behaviour. However, more recent evidence suggests a role for the dorsal striatum in GD (Boileau et al, 2014; van Holst et al, 2012b) as well as in irrational choice on the rBT (Cocker et al, 2012a). The OFC also appears to be implicated in the development of GD in response to DRT (Dagher et al, 2009; Frank et al, 2004; van Eimeren et al, 2009). Furthermore, our results in Chapter 5 showed changes in D2 receptors in the dorsal, but not ventral, striatum of rats following chronic ropinirole. Therefore, the next experiments
aimed to explore the involvement of the nigrostriatal pathway, which has strong connections with the OFC, in the development of gambling-like behaviours in rats following excitation or inhibition of this pathway with DREADDs.

The studies in Chapter 6 again showed that, reliably, chronic ropinirole increased preference for uncertainty in our population of male rats performing the rBT. However, that effect was unaltered by inhibition of the nigrostriatal pathway. In contrast, excitation of this pathway with DREADDs increased choice of uncertainty in our group of female wager-sensitive rats, somewhat mimicking the effect of chronic ropinirole.

7.2 Theoretical implications and considerations for future studies

Taken together, these results suggest that dopamine depletion in the nigrostriatal pathway is not necessary for the development of ICDs, implying that DRTs alone are strongly associated with the development of gambling-like behaviours in our animals. It is also likely that DRTs are independently responsible for inducing these side effects in the clinical population. The results also indicate that a prior tendency towards risky behaviours may not be a risk factor for the development of GD in response to DRTs. Although the experiments described here shed crucial light on the potential cognitive and neurobiological processes which may contribute to ICDs, there are still many critical considerations to observe.

7.2.1 Alternative neural substrates contributing to iatrogenic cognitive impairments

Our studies using excitatory DREADDs in the female rats also suggest a strong involvement for the nigrostriatal pathway in wager-sensitive rats, perhaps by increasing dopamine release in this pathway or by primarily influencing choice in animals that are relying on subjective, rather than objective, evaluation of reward to a greater degree when making
decisions on the rBT. Alternatively, excitation of the nigrostriatal pathway may have impaired learning from negative feedback, such that reward omission on the uncertain lever was less aversive in these animals. Similarly, chronic ropinirole may alter value-judgment on the rBT through affecting the OFC-dorsal striatum connectivity; inactivation of the OFC has a somewhat similar effect to chronic ropinirole, increasing preference for uncertainty on the rBT but only in wager-sensitive rats, matching the effect of exciting the nigrostriatal pathway with DREADDs (Barrus et al, 2016a). In contrast, ropinirole may not strongly affect more complex decision making on the rGT, which involves a larger neural network including the ventral mesolimbic system and BLA, but may instead affect motor impulsivity through this latter system. We also showed that the addition of cues to this task markedly increased the effect of the drug on motor impulsivity, possibly through its effect on the connectivity between the PFC and nucleus accumbens, given the implication of these brain areas in regulating impulsivity (Grace et al, 2007). Previous studies have shown that various structures important for valuation and reward processing, including subregions of the PFC and the BLA, are involved in performance of the uncued rGT (i.e. Pushparaj et al, 2015; Zeeb et al, 2015; Zeeb et al, 2013), and the role played by the OFC in mediating choice also changes depending on whether reward-predictive cues are included in a delay-discounting task (Zeeb et al, 2010). However, we did not see a strong effect of ropinirole on decision making in either the cued or uncued version of the rGT. Further studies will be needed in order to understand the contribution of ropinirole on impulsivity on this task, or in the implication of cues in potentiating the effect of ropinirole on impulsivity.
7.2.2 Molecular contributions to ropinirole-induced deficits in decision making

Rats in both the wager-sensitive and insensitive groups were affected by chronic ropinirole, but we saw a stronger effect of DREADDs excitation of the SNc on wager-sensitive rats. Further studies are needed in order to determine the difference in action between activation of the nigrostriatal pathway with DREADDs, which most likely acts on presynaptic release as compared to the effect of ropinirole, which would be expected to act on both the pre- and postsynaptic receptors. Perhaps, assessing the level of dopamine displacement associated with treatment with ropinirole or DREADDs activation through micro-PET imaging, as well as autoradiography to determine the degree of receptor distribution in the dorsal striatum, could help us understand the involvement of these receptors and dopamine release in the development of gambling-like behaviour and ICDs in response to DRT.

Our results from the western blot experiments, as well as from previous studies indicate that variation in levels of D2 receptor expression in the dorsal striatum may possibly confer vulnerability to gambling-like behaviours, or instead may be a consequence of chronic ropinirole. Further evidence for the importance of this area comes from studies in individuals with GD in which dopaminergic changes occurred in this area (i.e. Boileau et al, 2014; van Holst et al, 2012b). Given that ropinirole and pramipexole act at D2/3 receptors, it is not impossible that compensatory mechanisms in D2 receptor expression following chronic DRT may play a role in the development of ICD. In addition to the PET and autoradiography approaches discussed above, experiments that could determine the level of dopamine release in this area such as with microdialysis or voltammetry, prior to start of DRT, could help determine whether individual differences in dopaminergic tone or D2 receptor function represent a risk factor for the development of ICDs in response to DRT.
7.2.3 Assessing importance of sex differences on responsivity to DRT protocols

In the studies involved in this dissertation, we examined the effect of chronic ropinirole in populations of male rats. Our last experiment studied activation of the nigrostriatal pathway with DREADDs in female rats. It is well known that the dopamine system interacts in important ways with estrogens (Bourque et al., 2012; Gillies et al., 2014). Also, although men are more likely to suffer from PD, women also develop the disorder, and are more likely to suffer from restless leg syndrome, from which DRTs can also lead to the development of ICDs (Quickfall et al., 2007; Tippmann-Peikert et al., 2007). It will therefore be important to characterize the effects of chronic ropinirole in gambling-like behaviours on the rBT and rGT in female rats. Furthermore, although we did not think this may be the case, it is not impossible that the results observed in the wager-sensitive rats may be due to interaction with estrogens in these female rats. The duration of the estrous cycle is about 4 days (Marcondes et al., 2002), and animals would therefore have cycled multiple times during our chronic DREADDs activation manipulation. If the phase of the estrous cycle was dramatically impacting the response to ropinirole, we therefore should have seen some variation in behavioural response. Previous work suggests that the phase of the estrous cycle did not alter performance of a different risky decision making task, but females were more sensitive to amphetamine administration (Orsini et al., 2016). Since ropinirole and pramipexole are dopamine agonists, it is therefore not impossible that females are also differentially affected by these drugs. Consistently, future studies should also attempt to replicate the effect of activation of the nigrostriatal pathway in male rats.

Nonetheless, given that in the clinical population, both women and men may develop DRT-induced ICDs, the effect of these drugs appears to not discriminate between the sexes.
7.2.4 Alternative neurotransmitter involvement

We assumed an involvement of the dopamine system in the effect observed given that ropinirole acts predominantly as a D$_{2/3}$ agonist. However, the dopamine system interacts with multiple other systems such as the 5-HT system (Alex and Pehek, 2007; Kalivas and Volkow, 2005). It was also shown that repeated administration of D$_{2/3}$ agonist may desensitize both D$_{2/3}$ and 5-HT$_{1A}$ autoreceptors (Chernoloz et al, 2009). The studies in this dissertation did not assess the potential effect of chronic ropinirole on the 5-HT system. However, it is well known that this system is highly implicated in impulsivity, and could therefore be implicated in the development of ICDs (Crockett, 2009; Crockett et al, 2012; Crockett et al, 2010; Crockett et al, 2009; Leeman et al, 2013). Serotonergic drugs also affect choice behaviour on the rGT (Zeeb et al, 2009), and therefore, if ropinirole strongly affected this system, we would expect an effect of ropinirole on the rGT. However, this drug affected motor impulsivity on both the uncued and cued rGT, but did not affect choice performance of the uncued variant of the task, and the effect observed on the cued version of this task may reflect activation of D$_3$ receptors (Barrus et al, 2016b). However, here the change in choice behaviour on the cued rGT was not associated with increased choice of P3, as was seen with a D$_3$ agonist in the previous study mentioned. Nonetheless, future studies should characterize the contribution of the 5-HT system on performance of the rBT. Interestingly, 5-HT$_{2C}$ receptors seem to play a role in the treatment of impulsivity. For example, the 5-HT$_{2C}$ agonist lorcaserin was recently approved by the FDA for binge eating disorder, in which consumption of food is associated with high impulsivity and numerous studies suggest this drug class can decrease impulsive and addictive behaviours (Berg et al, 2008; Higgins and Fletcher, 2015; Iwamoto et al, 2009). In animals, systemic administration of a 5-HT$_{2C}$ receptor agonist decreased premature responding on the 5CSRT (e.g.
Navarra et al, 2008; Winstanley et al, 2005). Also, 5-HT\textsubscript{2C} agonists attenuate amphetamine-induced increase in premature responding (Fletcher et al, 2011), and reduced cognitive deficits observed in serotonin-depleted mice (Del'Guidice et al, 2014). In another study, the atypical antidepressant mirtazapine, which has 5-HT\textsubscript{2C} inverse agonist properties amongst other mechanisms, was partially successful in reducing pramipexole-induced increase in probabilistic discounting in rats (Holtz et al, 2016). Although the involvement of 5-HT appears to be more strongly related to motor impulsivity as opposed to more cognitive impulsivity, it is possible that 5-HT drugs may attenuate the ropinirole-induced increase in preference for uncertain outcomes on the rBT.

7.2.5 Potential genetic predictors of ICDs

Although we did not assess gene expression in our studies, a number of studies have attempted to determine potential predictive genetic factors in the development of ICDs in PD patients on DRT. For example, modifications in the OPRK1, HTR2A and DDC genes have been suggested as potential marker for ICDs in response to DRT (Kraemmer et al, 2016). Other allelic modification in the dopamine D\textsubscript{3} and glutamatergic receptor genes have also been suggested as potential risk factors. For example, the AA genotype of DRD3 p.S9G, a variant of the dopamine D\textsubscript{3} receptor, was associated with risk to develop ICDs in a population of PD patients (Lee et al, 2009). As mentioned, denervation of dopamine in the dorsal striatum in PD leads to expression of D\textsubscript{3} receptors on D1-expressing neurons of the direct pathway (Bordet et al, 1997). Also, gambling severity and impulsivity have been associated with greater D\textsubscript{3} binding in the substantia nigra of individuals with GD (Boileau et al, 2013), and these receptors have been implicated in the motivation to take drugs of abuse and in drug seeking behaviours (Sokoloff et al, 2017). Interestingly, drugs acting predominantly at this receptor have also been suggested to
increase the risk to develop ICDs (Seeman, 2015).

In addition, a variant of the glutamate N-methyl-D-aspartate (NMDA) receptor, the GRIN2B variant, is mainly expressed in the striatum and may also be involved in DRT-induced ICDs (Lee et al., 2009; Monyer et al., 1992). In support for this hypothesis, glutamate has been involved in the transition from reward seeking to more repetitive or habitual behaviour in addiction (Brewer and Potenza, 2008) and their number in the ventral striatum is associated with reward seeking (McFarland et al., 2003). Therefore, expression of various genes could contribute to the development of ICDs in response to DRT and act as potential predictive genetic factors. It would therefore be useful to identify which patients are at risk for developing these side effects and help improve treatment outcomes as well as help physician decide on a course of therapeutic strategies.

7.3 Critical considerations and limitations

On a statistical perspective, although we did correct for violation of the sphericity assumption revealed with Mauchly’s test using the Greenhouse-Geisser procedure, we did not assess for distribution normality in our data. Although, within-subject ANOVA testing is robust to violations of normality, it can overestimate significance in the case of highly skewed data, especially for between-subject testing. Due to the inclusion of individual differences in our analyses, and although we were consistent with previous literature in our field, future studies could test for normality using Shapiro Wilk or Kolmogorov-Smirnov Goodness-of-Fit test and correct for potential violation with non-parametric statistics that do not rely on normality assumptions.
In the second experiment in Chapter 3, we used the rat bilateral dorsolateral striatal 6-OHDA lesion model of PD, which features loss of dopamine terminals of about 25-30% in the dorsal striatum, as a proxy for early PD in older animals (Baunez et al, 2007; Blesa et al, 2012; Deumens et al, 2002; Przedborski et al, 1995). In PD, dopamine neurons in the SNc die. However, bilateral lesion to this area in rodents leads to severe motivational deficits (see Magnard et al, 2016 for review) Our need to keep animals able to perform complex decision-making tasks required us to use a model that did not lead to severe motor or motivational deficits and, therefore, we could not use the bilateral SNc lesions as a model for PD in the experiments included in this dissertation. As mentioned, although lesioned animals in our 6-OHDA model may show persistent forelimb motor impairment following striatal lesions, this model allowed performance of complex behavioural tasks such as performance of the rBT without development of side biases (Baunez et al, 2007; Blesa et al, 2012; Deumens et al, 2002; Przedborski et al, 1995; Rokosik et al, 2012). Although not optimal, this model was therefore chosen in Chapter 3.

In Chapter 6, we instead directly targeted dopamine release in the SNc with inhibitory DREADDs to model PD. As previously suggested, inhibition using this technique affects about 66% of the cells targeted (Gremel et al, 2013; Smith et al, 2016), and inhibition of these neurons reaches about 60% of their baseline activation (Chang et al, 2015). Therefore, both the bilateral dorsolateral striatal 6-OHDA lesion model and inhibition of dopamine release in the SNc neurons with DREADDs do not come close to the dramatic degeneration of 50-80% of dopamine neurons and terminals seen in the clinical population affected with PD at the time when the disease is diagnosed (Wilson et al, 1996). For these reasons, it is possible that the effect of ropinirole may have differed in models of more complete dopamine depletion. In addition, we assessed the extent of lesions in our 6-OHDA lesion model by immunohistochemistry. Future
studies could assess whether these lesions had altered the amount of dopamine released in the dorsal stratum by microdialysis.

Because of the symptomatic resemblance with the disease, as well as the similarity in inducing dyskinesia following L-DOPA treatment, the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model is widely used in animal models to attempt to understand the mechanism underlying both PD and dyskinesia. MPTP can cross the blood-brain barrier in mice and therefore models include single or multiple intraperitoneal administrations. Researchers have demonstrated that MPTP administration successfully diminishes the number of tyrosine hydroxylase immunoreactive cells in the striatum and substantia nigra seven days following a single administration of the toxin in animals (Jackson-Lewis and Przedborski, 2007). Also, increasing administrations and doses of the toxin increase the severity of neuronal loss (Di Monte et al, 2000). However, rats, as compared to mice, are much less sensitive to the neurodegenerative effects of MPTP and show a large degree of recovery following MPTP administration making this model not widely used in this rodent species (Giovanni et al, 1994a; Giovanni et al, 1994b; Schober, 2004). Animal models targeting genes involved in PD such as α-synuclein, LRRK2, or parkin, amongst others, could also offer alternatives to model this disease and study the effect of ropinirole on behaviour (i.e. Li et al, 2014; Lucking and Brice, 2000a; Lucking et al, 2000b).

Nevertheless, as mentioned previously, although ropinirole and pramipexole medications are primarily used in the treatments of motor symptoms associated with PD, they are also used for other disorders in which the dopamine system is unaffected such as restless leg syndrome and fibromyalgia (Holman, 2009; Quickfall et al, 2007; Tippmann-Peikert et al, 2007; Voon et al, 2011c). Although the incidence of ICDs is less frequent in these groups of patients, possibly to
the lower dose of drugs used, reports have shown that ICDs can also be observed following
initiation of DRTs in these populations. Consistent with our findings from dorsolateral lesions to
the striatum as well as from inhibition of dopamine release in the SNc, findings in the clinical
population indicates that alteration in brain neurochemistry is not necessary for the development
of DRT-induced ICDs, but instead that these side-effects may be attributable to DRTs
themselves. Although new evidence from the gambling literature points to the dorsal striatum as
a potential mechanism (Boileau et al, 2014; van Holst et al, 2012b), it is also possible that the
ventral mesolimbic system is involved (see Dagher et al, 2009; Napier et al, 2015; Voon et al,
2011b). Although previous studies did not observe an effect of basolateral amygdala lesion on
the rBT, it is possible that the ventral mesocorticolimbic pathway could be involved in other
forms of ICDs such as punding or even in different forms of gambling biases not assessed by the
rBT such as loss-chasing (Tremblay et al, 2014; Winstanley et al, 2016a). It has been shown that
phasic dopamine release in the ventral striatum is important in reward processing (Frank, 2005).
However, given that ropinirole increases dopamine in the brain through tonic activity, that we
observed a strong effect of the drug on the rBT, that activation of the SNr neurons on this task
led to somewhat similar effect to chronic ropinirole, and given the evidence for a role for the
dorsal striatum in GD, it is possible that persistent stimulation of D2 receptors with ropinirole in
this area impairs the decrease in phasic dopamine needed for negative feedback learning and
explain the effect observed on the rBT (Cohen et al, 2009; Dagher et al, 2009; Frank et al, 2004).

Ropinirole is an indolinone derivative metabolized primarily by the hepatic cytochrome
P450 CYP1A2 into the metabolites SKF-104557, SKF-97930 and SKF-96990 in humans and
SKF- 89124 in rats, excreted by the kidneys. These metabolites may have psychoactive effects
and could be responsible for the behavioural changes seen with chronic ropinirole. However, in
a study assessing the activity of ropinirole’s metabolites in a 6-OHDA model of PD and using radioligand binding and microphysiometer functional assays, it was concluded that the primary metabolites of ropinirole did not significantly contribute to its behavioural anti-parkinsonian properties (Reavill et al, 2000). Similarly, another study showed that the major human metabolite of ropinirole, SKF-104557, although also having some affinity for the D₄, along with the D₂/₃ receptors, had lower functional potency than ropinirole (Coldwell et al, 1999). Therefore, it is unlikely that the increase in preference for uncertainty observed on the rBT may be due to the effect of ropinirole’s metabolites. However, it is not impossible that there may be individual differences in the metabolizing capacity or rate of ropinirole which could potentially explain the variation in the strength of response.

In the clinical population, ropinirole can lead to a variety of side-effects ranging from dizziness, nausea, hallucinations, orthostatic hypotension and sudden sleep attacks. Although it is difficult to assess such effects in the rats, we do not believe that ropinirole led to such strong side-effects in our studies. First, the dose of ropinirole used here was low and slowly released throughout the day through osmotic release via a subcutaneous mini-pump. Also, we did not visually observe effects such as sudden sleep attacks in our rats. On the contrary, our rats appeared healthy and showed an increase in trials in our first study using the rBT, while also making fewer hole omissions and a reduced choice latency in most groups treated with ropinirole. If rats were experiencing dizziness or sleep attacks, we would expect to see a slowing in decision making while on the task, which we did not observe here.

Nonetheless, in these studies, we did not use a dose response curve in order to determine the dose of ropinirole to use in the osmotic pump. In a pilot study that was not included in this dissertation, we tried an acute dose of 1 mg/kg on the rBT, but this dose did not lead to
significant difference between the groups on any of the parameters of decision making tested. It is hard to extrapolate between rats and humans as to dose-ranging to be used as chronic delivery in an osmotic mini-pump, given the faster rate of metabolism in rats. As mentioned, the half-life of ropinirole is 5-6 hours in humans, but only 0.5 hours in rats (Ramji et al, 1999). In addition, acute doses used in previous studies in the range of 1-5 mg/kg in rats given chronically would most likely surpass the therapeutic range used in humans (Fukuzaki et al, 2000; Mavrikaki et al, 2014; Nashatizadeh et al, 2009). We therefore chose a dose that was similar to the single daily dose of the prolonged released formulation of ropinirole used in human patients (Nashatizadeh et al, 2009), but did not appear to lead to cognitive deficits (Matsukawa et al, 2007; Millan et al, 2004; Rogers et al, 2000). However, it could have been useful to perform a dose-response for chronic ropinirole administration perhaps by using an osmotic pump that delivers the drug for 7 days. It could be useful to determine whether we could reach the same behavioural change, a lower responder rate more similar to the human population, or if we could better control the behavioural effects with a lower dose of the drug. Alternatively, if individual differences in the rate of metabolism of ropinirole accounted for the lack of response in the non-responder rats, a higher dose of the drug could potentially tip these rats into becoming responders. Further studies could address this limitation by testing various doses of ropinirole in an osmotic pump.

Although the data described here have pointed to important contributors to the development of iatrogenic GD, there are some technical limitations to consider for future research. It can be argued that we used pulsatile delivery of CNO in our chronic DREADD-mediated activation and inhibition of the nigrostriatal pathways. Although speculative, it is possible that this more pulsatile administration regimen led to peak concentrations of the drug that may have different effect compared to a more consistent drug levels such as with chronic
ropinirole. A recent study has used CNO in an osmotic pump for 7 days with sustained efficacy (Donato et al., 2017), but to date, no studies to our knowledge have assessed the stability and efficacy of CNO in an osmotic minipump over a 28 day period. However, development of a subcutaneous CNO mini-pump protocol, if doable, would allow for a slower and more stable release of the drug. The lower concentration of CNO that would result from such a slow release formulation may also reduce the chances that any behavioural effect will be contaminated by metabolism of CNO into a bolus of clozapine. Furthermore, implantation of an osmotic pump delivering CNO would also reduce the need for daily restraint and injection of the rats, which would hopefully reduce stress for the animals.

Our Western blot analyses raised interesting questions as to the extent to which the GSK3β target kinase is involved in the effects of chronic ropinirole on the rBT. Although the Akt/GSK3β pathway may be involved in the response to continuous activation of D2/3 receptors with chronic ropinirole, we were not able to determine whether this activation contributes to the increase in preference for uncertainty on the rBT. While we confirmed, in two independent experiments, that chronic ropinirole increased levels of GSK3β in animals that increased choice of the uncertain option, we were not able to block this behavioural effect with repeated administration of a selective GSK3β inhibitor. However, we also were unable to block the ropinirole-induced increase in GSK3β with the GSK3β inhibitor, suggesting that either this drug was ineffective when administered chronically, or that it failed to cross the blood-brain barrier at sufficient concentrations to impact cell signaling. Our observation that the decrease in D2 receptors caused by chronic ropinirole could be reversed by the GSK3β inhibitor suggests that the latter may not be correct. Regardless, the action of the GSK3 kinase is complex (Liang et al., 2007; Zhang et al., 2003), and further understanding of the implication of this signaling pathway
on behaviour is necessary in order to definitively rule out its involvement in the effect of ropinirole. For example, GSK3 is constitutively active and is involved in many cell functions such as glycogen metabolism and cell cycle regulation. Inhibition of this kinase interact with other processes such as increasing the activity of β-catenin and can lead to unwanted adverse effects (O'Leary and Nolan, 2015). It is therefore believed that benefits from inhibition of GSK3 would be more likely in conditions where expression of this kinase is pathologically high. For example, studies suggest that GSK3 inhibitors such as SB 216763 can reduce neuroinflammation in pathological conditions in which GSK3 is elevated (De Sarno et al, 2008), but instead can stimulate glial response and neuronal damage in healthy systems (Hu et al, 2009). Although our healthy rats would not be expected to show aberrantly high levels of GSK3, elevation potentially due to chronic ropinirole administration and the change in behaviour associated with this drug could therefore benefit from GSK3 inhibition. Given concerns over the solubility or efficacy of the drug used, future studies should assay the involvement of the Akt/GSK3β pathway in ropinirole-induced gambling-like behaviours in rats using a GSK3β-KO model using the CRISPR/Cas9 technique. The difficulty with new compounds such as SB 216763 to cross the blood brain barrier at a high enough concentration to alter cognition has also been mentioned, along with the idea that GSK3 inhibitors may not be as effective in the treatment of cognitive dysfunctions related to neuropsychiatric disorders, and therefore may not be as effective for the treatment of ICDs (Eldar-Finkelman and Martinez, 2011; O'Leary et al, 2015).

Another consideration pertinent to all the studies conducted here is that rats were extensively trained on our behavioural tasks prior to the start of chronic ropinirole or excitation/inhibition with DREADDs. Previous work with the uncued rGT suggests that the neural circuitry responsible for acquiring the task can differ from that used to maintain choice
strategies following acquisition (Zeeb et al., 2011, 2013), time points in which the animals may be “exploring” the task’s contingencies versus “exploiting” strategies. This is a particularly important question when considering the involvement of the dorsal striatum in goal orientation and habitual behaviours after training. Given that the contingencies shift throughout each session in the rBT, and the order of blocks of trials of each bet size varies between sessions, it is less likely that choice patterns could become automatic or inflexible. Arguably, this may be more of a concern with respect to the rGT, in that the reinforcement contingencies remain constant throughout each session, and across training days. However, previous work indicates that choice patterns remain sensitive to reinforcer devaluation, and therefore remain broadly goal-directed. Nevertheless, it would be interesting to determine whether chronic ropinirole would alter choice if administered during acquisition of the rGT, or even of the rBT. In addition, we used rodent decision-making tasks with the goal of learning about potential aberrant decision making observed in humans with PG. However, tasks such as the IGT on which the rGT was based, was not designed to assess gambling behaviour, but instead was designed to assess everyday human decision-making styles (Bechara et al., 1999; Bechara et al., 1997). In comparison, pathological decision making observed in GD reflects biases in how individuals process uncertainty and rationalize risk (Cocker et al., 2015; Winstanley et al., 2016a). Therefore, although tasks such as the rBT and the rGT may not assess GD per se, they may be really useful to assess specific types of decisions making or biases analogous to those seen in the human clinical population.

Nonetheless, although the development of a more risky profile in PD patients on DRT may be seen as disadvantageous, we should also consider that there may also be advantages brought about by DRTs’ non-motor effects on behaviours. For example, we have mentioned
some detrimental effects of PD pathology such as the PD personality and symptoms such as depression, anxiety and apathy that may occur as the disease advances (Chaudhuri et al, 2006; Dagher et al, 2009; Todes et al, 1985). From this perspective, the effect of DRTs on generating a more risky profile could therefore be advantageous and useful in improving mood and apathy in those patients affected by these symptoms.

7.4 Concluding remarks

Here we showed that chronic ropinirole leads to dramatic and reliable increases in gambling-like behaviour on the rBT, similar to the effect of DRTs in PD patients, and in those with other disorders for which DRTs are prescribed. We also showed that the dorsal striatum may be involved in response to ropinirole and possibly in DRT-induced ICDs. Although further research is needed to understand the involvement of GSK3β in these behaviours, it appears that complex molecular mechanisms may account for the development of devastating ICDs, and that D₂ receptors in the dorsal striatum may be implicated.
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