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Influence of Pramipexole on Probability Discounting and Ventral Pallidal Function: Assessments in Parkinsonian-Like Rats

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INFLUENCE OF PRAMIPEXOLE ON PROBABILITY DISCOUNTING AND VENTRAL PALLIDAL FUNCTION: ASSESSMENTS IN PARKINSONIAN-LIKE RATS

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To Addison and Jackson

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LIST OF ABBREVIATIONS

6-OHDA	6-hydroxydopamine
ACC	anterior cingulate cortex
AMG	amygdala
BDNF	brain-derived neurotrophic factor
Camp	cyclic adenosine monophosphate
DMI	desipramine
DA	dopamine
D1R	dopamine D1 receptor
D2R	dopamine D2 receptor
D3R	dopamine D3 receptor
D4R	dopamine D4 receptor
D5R	dopamine D5 receptor
DLPFC	dorsolateral prefrontal cortex
DLS	dorsolateral striatum
ECur	effective current
ED	effective dose
FR	fixed ratio
GP	globus pallidus
IHC	immunohistochemistry

ICDs	impulse control disorders
ICSS	intracranial self-stimulation
ip	intraperitoneal
ISI	interspike interval
LR lever	large reinforcer/risky
LH	lateral hypothalamus
L-DOPA	levodopa
E_{max}	maximal effect
MFB	medial forebrain bundle
GPm	medial globus pallidus
NA	nucleus accumbens
OFC	orbitofrontal cortex
PD	Parkinson's disease
PD-ICD	Parkinson's disease patients with impulse control disorders
PPX	pramipexole
PPN	pedunculopontine nucleus
PFC	prefrontal cortex
rm	repeated measures
RLS	restless leg syndrome
SC lever	small reinforcer/certain
sc	subcutaneous
SNpc	substantia nigra pars compacta
SNpr	substantia nigra pars reticulata

STN	subthalamic nucleus
TH	tyrosine hydroxylase
VP	ventral pallidum
vSub	ventral subiculum
VTA	ventral tegmental area
VLPFC	ventrolateral prefrontal cortex
VMPFC	ventromedial prefrontal cortex

CHAPTER I

INTRODUCTION

People with neuropathologies that are treated with dopamine (DA) agonists may be at risk to develop impulse control disorders (ICDs). ICDs are defined as a group of psychiatric disorders characterized by a failure to resist an impulse, drive, or temptation to perform an act that is harmful to the individual or to others. In North America, they most commonly occur in the form of pathological gambling, hypersexuality, excessive shopping, or excessive eating. The first published reports of this phenomenon came from Parkinson's disease (PD) patients. Reports in restless leg syndrome (RLS) patients then followed. Speculation regarding the particular drugs most responsible for these behaviors included Requip® (ropinirole) and Mirapex® (pramipexole; PPX). Both drugs are direct DA receptor agonists that demonstrate a preference for the DA D3 receptor (D3R) over the DA D2 receptor (D2R). PPX was FDA approved for PD in 1997 and for RLS in 2006 and currently is being used off label for other pathologies including major depression, fibromyalgia, and bipolar disorders. In these latter two conditions, case reports about patients developing PPX-induced pathological gambling have appeared. Thus, once thought to be a phenomenon to the PD population, ICDs are now evident in several other neuropathologies. The idea that properties of the DA agonists themselves can influence impulsive behavior is gaining acceptance as clinical studies using healthy

controls demonstrate that drugs such as PPX can shift reward-based learning towards increased risk-taking. Although it appears that the properties of these DA agonists are contributing to ICDs, it does not preclude the possibility that a brain state, such as that seen in PD, makes the individual more vulnerable.

At the time this dissertation was being developed there was a struggle for some people to accept these therapy-induced ICDs as a true phenomenon. Common questions arose. Was the incidence of ICDs greater in PD than in the general population? How could a drug used for motor complications be causing individuals to throw away their life savings at a casino, or increase their sexual desires? Given the anecdotal evidence at the time, there was a clear need to develop preclinical animal models to study the neurobiology, pharmacology, and behavioral effects of these drugs.

The ***overall goal*** of this thesis dissertation project was to expand our knowledge on the neuropsychopharmacology of DA agonist-induced impulsivity. At the time this dissertation was being developed, PPX was the drug, pathological gambling was the behavior, and PD was the pathology most widely reported for this phenomenon. Therefore, we first developed a behavioral paradigm (i.e., a probability discounting) to measure risk-taking, one aspect of gambling. Next, we utilized this paradigm to determine if risk-taking was altered after acute and/or chronic PPX treatment. We incorporated an animal model of PD in this study to determine if a PD-like brain state alters the response of PPX in the discounting paradigm. The final series of studies focused on determining if a limbic brain region that is involved in reward-related behaviors is also altered by acute and chronic PPX exposure. Prior work from our laboratory and others suggest that the ventral pallidum (VP) would be a region of interest.

The VP mediates animals' responses to rewards and VP neural activity integrates predictive, incentive, and reward value information. As studies show that PPX can alter aspects of impulsivity, such as risk-taking, as well as enhance the motivational salience of reward-related cues, it is possible that the VP plays a role in mediating these effects of PPX. Accordingly, we hypothesized that VP neuronal activity is altered by behaviorally relevant doses of PPX. To test this hypothesis, we utilized single cell extracellular electrophysiological techniques to investigate the effects of systemic PPX on VP neuronal firing rate. Finally, as D3Rs can mediate reward-seeking behavior, we investigated the influence of D3Rs in the ability of PPX to alter VP neuronal firing rate using PG01037, one of the most D3R-selective antagonists available to date. Collectively, my studies demonstrate that acute administration of PPX treatment enhances risk-taking in rats and also modulates VP neuronal firing rate, this modulation appears to be mediated by D3R activation. Chronic treatment with PPX enhances risking-taking compared to acute treatment. Chronic treatment also enhances the potency of PPX to alter VP neuronal firing rate. Finally, these studies suggest that a PD-like brain state does not alter PPX-induced alterations in risk-taking.

CHAPTER II

LITERATURE REVIEW

Parkinson's disease and dopamine replacement therapy

Parkinson's disease

PD is classically considered a movement disorder that is characterized by a significant loss of DA in the dorsal striatum and loss of neurons in the nigrostriatal pathway (Fearnley and Lees, 1991; Bernheimer et al., 1973). When the cardinal motor signs present (bradykinesia, resting tremor and rigidity) approximately 60% of dopaminergic nigral neurons are lost and striatal DA content is reduced by 60-80% (Tissingh et al., 1998). An understanding of the motor pathways that are influenced by the dopaminergic nigrostriatal system can help explain how movement is affected in PD.

As diagrammed in Fig. 1, a popular model of the motor circuitry of the basal ganglia (Albin et al., 1989; Wichmann and DeLong, 1993) involves "direct" and "indirect" pathways. Both pathways originate in the striatum (caudate putamen) and converge on the same motor output structures (i.e., medial globus pallidus (GPm) and the substantia nigra pars reticulata (SNpr)). DA released from the substantia nigra pars compacta (SNpc) is able to both excite and inhibit striatal neurons; this modulation is thought to occur via DA activating D1-like receptors and D2-like receptors, respectively. In general, D1-like family of receptors (which includes the D1 and D5 subtypes) are

highly localized in striatal neurons that project directly to the GPm/SNpr, whereas D2-like family of receptors (which includes the D2R, D3R and D4R subtypes) are highly localized in striatal neurons that project to the GPm/SNpr *via* the lateral globus pallidus (GPl) and subthalamic nucleus (STN) (Gerfen et al., 1990; Surmeier et al., 1996; Gong et al., 2003). DA-induced activation of the direct pathway and inactivation of the indirect pathway can have opposite effects on motor behavior (Kravitz et al., 2010). Thus, in general, DA acting on D1Rs in the direct pathway facilitates movement; DA acting on D2Rs in the indirect pathway inhibits movement.

Several mechanisms by which DA modulates (i.e., increases or decreases) the activity of striatal neurons have been elucidated. For example, DA can modulate the intrinsic excitability of striatal neurons. At rest striatal neurons are held in a hyperpolarized state and glutamatergic input from the cortex can depolarize the membrane potential of these striatal neurons (Shen et al., 2007). Activation of D2Rs diminishes, while activation of D1Rs supports the ability of glutamate to depolarize the neuron (Surmeier et al., 2007). DA can also modulate the ion channels that influence neuronal firing. For example, activation of striatal D2Rs in the indirect pathway can inhibit neuronal activity by suppressing calcium currents (Hernandez-Lopez et al., 2000). D1R activation can excite neuronal activity by enhancing L-type calcium currents (Hernandez-Lopez et al., 1997). These, plus other mechanisms are thought to allow nigrostriatal DA to modulate motoric behavior.

Recently, a third ‘hyperdirect’ pathway was incorporated into this circuit model which is a cortico-STN-pallidal pathway (for review, see (Nambu et al., 2002)). The term ‘hyperdirect’ refers to the observation that the signal conduction time from this

pathway is faster than that observed in the direct and indirect pathway (Nambu et al., 2000). The hyperdirect pathway exerts powerful excitatory effects on the GPm/SNpr and, similar to the indirect pathway, its activation results in an inhibition of the thalamic motor nuclei and motor cortex.

In PD, nigrostriatal dopaminergic degeneration induces a decrease of striatal inhibition *via* the direct pathway and an increase in subthalamic activation *via* the indirect pathway, both of which result in hyperactivity of the GPm/SNpr (see Fig. 2). The increase of inhibition exerted by the GPm/SNpr on the motor thalamo-cortical projections leads to inhibition of motor cortical areas, resulting in an inhibition of movements, seen as bradykinesia in PD.

The ability of DA agonists to not only improve motor but also affect decision making has been linked to the indirect and direct pathway. Frank and colleagues have generated computational models that use these direct pathway (i.e., the 'GO' pathway) and the indirect pathway (i.e., the 'NoGo' pathway) to explain how reinforcement learning can influence decision making both in a PD-like brain state and with DA agonist treatment (Frank et al., 2004). This will be discussed in further sections.

Dopamine replacement therapy

DA replacement therapy is used to treat the motor symptoms in PD. Common therapies include the indirect DA agonist, levodopa (L-DOPA), and the direct DA agonist, PPX. L-DOPA was first introduced as a therapy for PD in the 1960's. In the brain, L-DOPA is taken up by surviving neurons and converted to DA by the enzyme DOPA decarboxylase. DA is then stored and, upon neuronal activation, is released. DA

acts on two different G-protein coupled receptor families, the D1Rs and D2Rs. As mentioned above, the D1 family includes the D1R and D5R subtypes; these are coupled to the Gs protein, and can activate adenylyl cyclase and thus increase intracellular concentrations of the second messenger cyclic adenosine monophosphate (cAMP). The D2 family includes the D2R, D3R, and D4R subtypes that are coupled to the $G_{i/o}$ protein and inhibits the formation of cAMP by inhibiting adenylyl cyclase (Spano et al., 1978; Kebabian and Calne, 1979). In PD, the imbalance of the direct pathway and the indirect pathway is thought to be rectified by DA synthesized from L-DOPA. Over time, however, its continued use leads to dyskinesias or abnormal involuntary movements (Marsden and Parkes, 1977) which can be more debilitating than the movement disorder caused by the disease itself. During L-DOPA therapy, motor response fluctuations also occur throughout the day, alternating between "ON" and "OFF" periods. In the "ON" periods, L-DOPA is working optimally, during "OFF" periods the patient is slow, rigid, tremulous, and dystonic. Therefore, due to some of the shortcomings of L-DOPA treatment, other DA replacement therapies alone, or in combination with L-DOPA are used.

PPX was FDA-approved for PD treatment in 1997. The half life of PPX in humans is 8-12hr (Wright et al., 1997), whereas in rats, it is estimated to be approximately 4hr (Ferger et al., 2010; Panchal et al., 2010). PPX is a direct DA receptor agonist that functions *via* activation of the D2-like receptor family, with highest affinities for D3Rs. Examples of reported K_is for PPX are as follows: D3R, 0.9nM; D2R, 6.9nM; D4R, 15nM; D1R >1,000nM (Piercey et al., 1996). PPX has a low affinity for α2-adrenoceptors and negligible affinities for other adrenergic, histaminergic, serotonergic,

cholinergic, glutaminergic, adenosine, and benzodiazepine receptors (Piercey, 1998). Collectively, studies show that PPX has a preference for the D3R over the D2R; however, there are discrepancies regarding the degree of selectivity of PPX for these two receptors between *in vivo* and *in vitro* assays. *In vitro* studies, using binding assays and mitogenesis assays, report PPX to be 5 to 170-fold more selective for D3Rs compared to D2Rs (Mierau et al., 1995; Sautel et al., 1995; Perachon et al., 1999; Newman-Tancredi et al., 2002).

The Woods laboratory (Collins et al., 2009; Collins et al., 2007; Collins et al., 2005) and others (Yamada and Furukawa, 1980) have demonstrated that various dopaminergic drugs, including PPX, induce yawning in rats (which is driven in part by DA receptor stimulation in the paraventricular nucleus of the hypothalamus (Mogilnicka and Klimek, 1977; Holmgren and Urba-Holmgren, 1980; Argiolas et al., 1989). They show that DA agonists produce an inverted U shaped dose-response curve. Lower doses increase while higher doses decrease the number of yawns per minute. Activation of D3Rs drives the initial ascending arm of the curve while D2Rs drive the latter descending part of the curve (Collins et al., 2005). Using this behavioral assay, the Woods laboratory determined that PPX-induced yawning at doses up to 100μg/kg are D3R selective, while higher doses activate D2Rs (Collins et al., 2009; Collins et al., 2007; Collins et al., 2005). They also determined that PPX is ~30 fold selective for D3R over D2R (Collins et al., 2007). These discrepancies in selectivity between *in vivo* and *in vitro* may be due to the differences in species, expression systems, radioligands, and/or assay conditions (Collins et al., 2007). Regardless of these discrepancies, it is clear that PPX is acting as a direct

agonist at both D2R and D3Rs. It is this receptor activation profile that is believed to be contributing to both motor and reward-related effects of PPX.

D2Rs are located throughout the nigrostriatal and mesolimbic systems, whereas D3Rs are relatively less abundant and restricted more to the mesolimbic regions compared to the nigrostriatal pathway (Bouthenet et al., 1991; Diaz, 1995; Diaz, 2000; Levant, 1997; Sokoloff, 1990; Landwehrmeyer, 1993a; Landwehrmeyer, 1993b; Meador-Woodruff, 1994). D2R and D3R are located both postsynaptically and presynaptically. Presynaptically, they function as autoreceptors that affect DA synthesis, release and signaling. The ability of PPX to alleviate PD symptoms is thought to lie in its ability to directly stimulate postsynaptic receptors in the striatum. The mechanism of action for PPX to improve motor impairments in PD is unknown. However, PPX has been proposed to simultaneously excite the "direct" pathway (by D3R stimulation) and inhibit the "indirect" pathway (by D2R stimulation). As explained by Piercey (1998), in the caudate, neurons can be characterized as type I or II based on the waveform of their action potentials. PPX, acting on D3Rs, can excite type II caudate neurons (Piercey et al., 1997) assumed to be in the "direct" pathway. At least three studies provide evidence that D3Rs can be expressed in the direct pathway. First, D3Rs colocalize with D1Rs on substance P/dynorphin-containing neurons (Surmeier et al., 1996). Second, DA denervation of the striatum (i.e., a 6-OHDA-induced lesion to the medial forebrain bundle (MFB) can lead to D3R expression on D1-expressing neurons (Bordet et al., 1997). Third, PPX-induced activation of type II caudate neurons can inhibit neuronal activity in the SNpr (Hoffman et al., 1996) which is a predicted outcome of activation of the direct pathway (see Fig. 1). The ability of D3Rs to *activate* the striatal neurons seems

counterintuitive since this receptor is G_i linked. However, D1R and D3Rs can form heterodimers in the striatum (Marcellino et al., 2008). In vitro studies show that chronic stimulation of the D3R with PPX leads to a sensitized activation of adenylyl cyclase (Maggio et al., 2009) suggesting that this could lead to activation of the direct pathway via the D1R associated Gs-protein. Therefore, it appears that PPX improves motor deficits by activation of striatal D2Rs and D3Rs which re-establishes a balance in the basal ganglia direct and indirect pathways.

Introduction to impulse control disorders

Although beneficial for motor symptoms, DA replacement therapies such as L-DOPA and PPX can induce maladaptive reward-related behaviors in some individuals. One of the most detrimental side effects includes ICDs. ICDs have been described as "behavioral addictions" (Grant et al., 2010) and are defined by a "failure to resist an impulse, drive or temptation to perform an act that is harmful to the person or others" (American Psychiatric Association, 2000). DA agonist-induced ICDs are diverse and culturally based (Kim et al., 2012). In North America, they commonly include problem/pathological gambling, hypersexuality, compulsive shopping and binge eating (Weintraub et al., 2010). The Dominion Report, a cross sectional study which included over 3000 PD patients, reported that ICDs occur in approximately 14% of PD patients; pathological gambling was found in 5% of the patients (Weintraub et al., 2010). Life time prevalence of pathological gambling in the North American general population is estimated at 1-2% (Shaffer and Hall, 2001). The Dominion Report provides evidence that ICDs can develop directly from agonist treatment.

Impulsivity can be regarded as “actions that appear poorly conceived, prematurely expressed, unduly risky, or inappropriate to the situation” (Daruna and Barnes, 1993). While at times impulsivity can be a beneficial character trait, (Dickman, 1990), it is generally recognized as a dysfunctional trait that is frequently associated with numerous neurological and psychiatric disorders including frontal lobe damage, schizophrenia, attention deficit-hyperactive disorder, substance abuse disorders and behavioral addictions, such as pathological gambling. According to the American Psychiatric Association, ICDs are a form of psychiatric disorder (American Psychiatric Association, 2000).

In the 1970’s, there were some case reports of hypersexuality in patients treated with L-DOPA (Ballivet et al., 1973; Bowers, Jr. et al., 1971; Wodak et al., 1972), but over the years little attention was given to this issue (Harvey, 1988; Jimenez-Jimenez et al., 1999; Vogel and Schiffter, 1983). When cases of ICDs re-emerged in the 2000’s (Seedat et al., 2000; Molina et al., 2000), relatively fewer case reports linked L-DOPA use to ICD’s (Molina et al., 2000) as compared to the direct DA agonists. Indeed, the Dominion Report revealed that ICDs were more common in patients treated with a DA agonist than in patients not taking a DA agonist (Weintraub et al., 2010). They reported that an ICD was present in 17.7% of patients taking both a DA agonist and L-DOPA, and in 14.0% taking a DA agonist without L-DOPA. These data clearly point to a role of DA agonists, and not L-DOPA, in medication-induced ICDs.

Rather than ICDs, L-DOPA monotherapy has been linked to DA dysregulation syndrome (a.k.a., hedonistic homeostatic dysregulation), a condition in which the patient takes their DA replacement therapy in excess of their therapeutic requirements (Lawrence

et al., 2003). Their medication use is maladaptive in so much as they self-escalate their doses even in the face of adverse consequences, which can include disabling dyskinesias, mood disorders and complex stereotypies (Lawrence et al., 2003). It is estimated that up to 4% of PD patients develop a DA dysregulation syndrome (Lawrence et al., 2003).

Epidemiological studies have characterized several vulnerabilities related to the occurrence of ICDs in PD patients. These include male gender, younger age, younger age at PD onset, a pre-PD history of ICD(s), personal or family history of substance abuse, and general impulsive personality traits (Voon et al., 2007; Weintraub et al., 2010). Lacking are reports that detail the onset of ICDs once DA agonist treatment is started, although one case study reported that in some patients onset occurred within 3 months of starting or escalating the dose of agonist (Dodd 2005). The primary management of ICDs in PD is discontinuation of DA therapy. The urges associated with ICDs can dissipate within days to weeks, and some patients are aware of this quite suddenly after a dose reduction (Macphee, personal communication). Not all patients can tolerate this, however, due to worsening motor symptoms and/or a DA withdrawal syndrome (Pondal et al., 2012; Rabinak and Nirenberg, 2010).

Impulse control disorders and reward-related learning

ICDs such as pathological gambling are fundamentally a breakdown in the decision-making process. Reward based decision-making is critically dependent on learning about rewards. I would like to discuss at least three forms of learning that are necessary to help an individual make appropriate reward-based decisions which I think are critical in the development of ICDs, particularly pathological gambling. The first

form of learning is a general reinforcement learning strategy that is based on trial and error. During this type of learning, an individual can generate predictions about whether or not a reward is going to be present, when the reward will be delivered, and how large or small the reward will be. Depending on the learned expectations of the reward, the individual can appropriately decide if it is worth seeking out. Positive outcomes, such as novel rewards, can generate seeking behavior, while negative outcomes, such as omission of rewards, can generate inhibition of behavior. In ICDs, it is thought that reward omissions are not properly processed, therefore learning from negative outcomes is impaired. The consequence is aberrant decision making. A second form of learning is reversal learning. In this case, an individual can inhibit a behavior that is no longer reinforcing and switch to a different behavior. Such behavioral flexibility is critical when reward-related conditions are changing. If the ability to learn a new rule changes or the ability to inhibit a response is impaired, the individual may continue to perform a behavior that once was, but no longer is, reinforcing. These two forms of learning are critical in guiding behavior that can lead to maximizing rewards. These forms of learning are particularly critical for maximizing rewards in a probability discounting task. This is a task that is used to measure risk-taking, on aspect of impulsivity, and a prominent behavior in ICDs such as pathological gambling. In a probability discounting task, a subject chooses between a small reward that is always delivered and a large reward that is delivered with varying probabilities. Risk-taking is defined as a preference for the large, uncertain reward. Learning about reward predictions is critical in this task. When the probability of delivery of the large reward is high, it is optimal for the individual to choose the large reward over the small reward. However, when the probability of

delivery of the large reward is low and choosing that option continually leads to no reward, it optimal to switch and choose the smaller, guaranteed reward. Inherent to optimizing rewards is the ability to demonstrate reversal learning (i.e., the ability to make the switch from selecting a previously large reward to now selecting a small, but certain reward).

A third form of learning is reward-mediated associative learning. Learning about cues that are associated with rewards is critical because those cues can predict the availability of the reward. As mentioned before, ICDs have been compared to drug addictions where behaviors are continued, even in the face of adverse consequences (Brewer and Potenza, 2008). Through associative learning, cues that have become linked to rewards can acquire salience and the cues can then act as powerful reinforcers that motivate the individual to seek out rewards and related cues, thus driving the addiction. Abnormal learning about rewards/cues could influence decision-making such that reward-seeking becomes the focus of the individual and self-control and behavioral inhibition are compromised. This behavioral profile is consistent with impulsive-like behaviors. In rats, D2/D3R agonists, including PPX can influence associative learning (Riddle et al., 2012; Collins et al., 2011) and reversal learning (Haluk and Floresco, 2009). In PD patients, PPX can disrupt reward-related learning in probability reversal learning task; it appears that learning by negative outcomes is impaired (Cool et al., 2001). In this dissertation we employed a probability discounting task to test our hypothesis that PPX would increase risk-taking behavior in rats.

Impulse control disorders and the related neurocircuitry

Brain regions that can mediate the three forms of learning discussed above are located in the limbic regions of the brain. I will discuss each of these brain regions and their contribution to decision making (see Fig. 3 for a schematic of these brain regions).

Dopaminergic neurons in the VTA can fire in a slow 'tonic' pattern or in a bursting 'phasic' pattern (Grace and Bunney 1984). The tonic firing supplies a baseline level of extrasynaptic DA in postsynaptic structures and in general activates D2Rs (Grace, 1991), whereas the phasic firing releases a transient increase in DA thought to be restricted in the synaptic cleft and is thought to act on D1Rs (Grace, 1991). These firing patterns encodes reward prediction errors (Montague et al., 1996; Schultz et al., 1997; Pagnoni et al., 2002; Pessiglione et al., 2006; Cohen et al., 2012; Enomoto et al., 2011). For example, some of these neurons will fire in a phasic pattern when an unpredicted reward is encountered (i.e., a positive reward prediction error; Hollerman et al., 1998; Waelti et al., 2001). On the other hand, some of these neurons will show a transient depression in baseline rate of activity, thought to be a pause in the spontaneous 'tonic' firing (Grace and Bunney 1984), when an expected reward is not delivered (Schultz, 2002; Bayer et al., 2007; Tobler et al., 2003). This results in a transient decrease in tonic release of DA to output structures and has been referred to as a 'DA dip'.

Many of the VTA output structures are also implicated in influencing risky decision making. These include the NA, AMG, and the VP. DA released from the VTA onto the NA is critically involved in the reinforcing and motivational effects of natural and drug rewards (Wise and Bozarth, 1981; Koob, 1996). DA signaling in this mesoaccumbal pathway plays an important role in response selection, behavioral

flexibility, associative learning, and risk-taking behavior as measured in probability discounting paradigms (Salamone et al., 1997; Ikemoto and Panksepp, 1999; Cardinal and Howes, 2005). The NA can also generate prediction errors, (Sugam et al., 2012; Schultz et al., 1992). The AMG processes stimulus–reward associations, particularly emotional responses. This is critical for the role of the AMG in associative learning (Kentridge et al., 1991), reversal learning (Stalnaker et al., 2009; Churchwell et al., 2009), and prediction errors (McNally et al., 2011). The AMG also plays a role in risk-taking as measured in a probability discounting task (St Onge et al., 2012). The hippocampus computes spatial and contextual information (Stubley-Weatherly et al., 1996) that is important for associative learning (Shen et al., 2006), and novelty learning (Cooper et al., 2001; Legault and Wise, 2001). The ventral pallidum (VP) is a critical substrate in associative learning (Dallimore et al., 2006; Mickiewicz et al., 2009; Gong et al., 1996). It encodes predictive and motivational information about rewards and their associated stimuli (Tindell et al., 2004;Tindell et al., 2005). The VP also encodes expected reward values (Tachibana and Hikosaka, 2012). Additionally, the VP has a direct influence over tonic spiking activity of dopaminergic neurons in the VTA (Floresco et al., 2003), thus placing it in position to modulate the response of VTA to rewards. The subthalamic nucleus (STN) is another brain region that mediates decision making. Historically associated with the movement and the basal ganglia, the STN is now known to influence impulsivity (Bogacz et al., 2012) as well as mediate reversal learning (El Massioui et al., 2007). Finally, the pedunculopontine nucleus (PPN), which is located in the brainstem, is a region that encodes reward prediction errors (Kobayaski and Okada, 2007) and is involved in associative learning (Bortolanza et al., 2010). The

PPN can directly regulate the phasic firing of dopaminergic neurons in the VTA (Floresco et al., 2003), thus placing it in position to modulate the response of VTA to rewards.

The PFC plays a critical role in mediating self-control. The PFC can be divided into several different subregions, with each controlling different aspects of decision-making. There are three regions that are considered particularly relevant for risk-taking and pathological gambling (see Fineberg et al., 2009). These include the orbitofrontal cortex (OFC), the ventromedial PFC (VMPFC) and the anterior cingulate cortex (ACC). Damasio, Bechara and colleagues revealed that the OFC mediates risk-taking as measured in an Iowa gambling task (Bechara et al., 1994; Bechara et al., 1999). The OFC is also involved in reversal learning (Ragozzino, 2007). Damage to the ventrolateral PFC (VLPFC) results in a blunted reaction to aversive outcomes as well as risk-taking behavior (Floden et al., 2008). The ACC mediates response-inhibition and reversal learning (Kerns et al., 2004; Kosaki and Watanabe 2012). The ACC also encodes reward prediction errors and is speculated to evaluate the consequences of choices made (Wallis and Kennerley, 2011). Collectively, all of these regions work together to evaluate reward-relevant contextual information from the environment and evaluate expectations of rewards so as to make decisions on how to execute behavior. Clinical studies demonstrating how these brain regions are affected in aberrant decision making are discussed below.

The ventral pallidum and its role in reward-meditated behaviors

In this section, I will expand on the importance of the VP as a mediator of reward-related behaviors. The VP is located at an interface between the mesolimbic system and the nigrostriatal system. A schematic of these brain regions are shown in Fig. 4. The VP receives projections from the NA (Groenewegen et al., 1993; Nauta et al., 1978; Chrobak and Napier, 1993), AMG (Krettek and Price, 1978; Bayer et al., 2007; Leonard and Scott, 1971; Mitrovic and Napier, 1998; Maslowski-Cobuzzi and Napier, 1994), PFC (Delgado-Martinez and Vives, 1993; Sesack et al., 1989), STN (Turner et al., 2001; Groenewegen and Berendse, 1990), and VTA and SNpc (Maslowski-Cobuzzi and Napier, 1994; Mitrovic and Napier, 2002; Klitenick et al., 1992). The VP projects to the NA (Churchill and Kalivas, 1994; Hakan et al., 1992), STN (Maurice et al., 1997; Bell et al., 1995), PFC (Sesack et al., 1989), VTA (Groenewegen et al., 1993; Kalivas et al., 1993), SNpr (Maurice et al., 1997), and brainstem targets including the PPN (Tsai et al., 1989). Based on these inputs and outputs of the VP, it is in a critical position to integrate limbic-processed reward information and influence final motor activation (Mogenson et al., 1980).

Studies highlight a role for the VP in reward and addiction. For example, the VP mediates food reward and consumption (Cromwell and Berridge, 1993; Berridge, 1996; Stratford et al., 1999), conditioned place preference (Dallimore et al., 2006; Mickiewicz et al., 2009; Gong et al., 1996), and drug self-administration (Tang et al., 2005; Caille and Parsons, 2004). Furthermore, the VP supports ICSS (Panagis et al., 1995). Critical information about rewards is encoded in VP neuronal firing activity. For example, the VP encodes predictive and motivational information about rewards and their associated

cues (Tindell et al., 2004; Tindell et al., 2005). The VP also encodes expected reward values (Tachibana and Hikosaka, 2012). Indeed, rodent and human studies demonstrate that the VP is activated by reward cues (Tindell et al., 2009; Mahler and Aston-Jones, 2012; Tsurugizawa et al., 2012; Childress et al., 2008). A neuroimaging study detected a positive correlation between activation of the VP following appetizing food cues and 'reward drive' (Beaver et al., 2006). Activation of the VP was also detected during a human functional MRI study in which there was increased motivational behavior in response to cues that predicted the potential gain of a large quantity of money (Pessiglione et al., 2007).

D2/D3R agonists, such as quinpirole, can influence VP neuronal activity (Napier and Maslowski, 1994; Maslowski and Napier, 1991; Napier, 1992). Therefore, it is predicted that PPX will also influence VP neuronal activity. The VP is a dopaminergic receptive structure (Napier et al., 1991; Napier and Potter, 1989; Klitenick et al., 1992). Although the DA innervation is sparse (Fallon and Moore, 1978; Klitenick et al., 1992), 50-70% of VP neurons are responsive to local application of DA, with similar proportions of firing rate-enhancements and suppressions observed (Johnson and Napier, 1997; Napier and Maslowski, 1994; Mitrovic and Napier, 2002; Napier and Potter, 1989; Napier et al., 1991). A majority of VP neurons are sensitive to electrical activation of the VTA and SN (Maslowski-Cobuzzi and Napier, 1994; Mitrovic and Napier, 2002). Pharmacological assessments of VP neuronal activity indicate that both D1Rs and D2Rs are expressed in the VP (Maslowski-Cobuzzi and Napier, 1994; Napier and Mitrovic, 1999). Using microiontophoresis techniques that allow application of D1R and D2R agonists within the vicinity of individual recorded VP neurons, spontaneously firing of

VP neurons is decreased by D1R agonists while D2R agonists increase firing rates (Napier and Maslowski, 1994). When these agonists are delivered systemically the opposite effects are seen. That is, D1R agonists increase VP neuronal firing rate; whereas D2R agonists produce rate decreases (Maslowski and Napier, 1991; Napier, 1992; Heidenreich et al., 2004; Heidenreich et al., 1995). These results indicate that VP neuronal activity is influenced by direct and indirect circuit related effects. D3R are also expressed in the VP as well as in the related circuitry including the NA, AMG, PFC, VTA, SN, and STN (Tziortzi et al., 2011; Murray et al., 1994; Flores et al., 1999; Bouthenet et al., 1991; Diaz et al., 1995; Diaz et al., 2000; Levant, 1997; Sokoloff et al., 1990; Stanwood et al., 2000a; Stanwood et al., 2000b). Moreover, DA acts as a modulator within the VP. For example, stimulation of the VTA attenuates glutamate-evoked responses in the VP that are induced by AMG stimulation (Maslowski-Cobuzzi and Napier, 1994). Also, co-application (using microiontophoresis) of DA with either glutamate or GABA substantially alters both GABA- and glutamate-evoked VP responses (Johnson and Napier, 1997). Given the direct and indirect effects of DA on VP neuronal firing, its role in associative learning and drug seeking behavior, its ability to encode predictive and motivational information about rewards and their associated cues and its ability to influence tonic DA activity in the VTA, we hypothesize that the VP is involved in mediating PPX-induced impulsivity.

Pramipexole-induced alterations in reward-related behavior in clinical settings

Following the numerous case reports of DA agonist-induced ICDs, clinical laboratories began investigating the ability of PPX to influence decision making. An

initial report from the de Wit laboratory suggested that PPX did not influence a variety of measures of impulsivity (Hamidovic et al., 2008); however, other studies in healthy subjects (Pizzagalli et al., 2008; Riba et al., 2008) as well as in RLS and PD patients (without ICDs) (Abler et al., 2009; van Eimeren et al., 2009; Cools et al., 2006; Bodi et al., 2009) demonstrated that PPX alters reward-based decision-making. For example, Riba (2008) demonstrated that PPX induces riskier choices following unexpected high wins in healthy subjects. Cools (2006) demonstrated that PD patients are impaired in a probabilistic reversal learning task when reversals were signaled by unexpected punishment. The difference between the negative findings in the Hamidovic study and the latter studies that showed a PPX-induced effect can possibly be explained by the tasks used (i.e., the latter studies all generally used a probabilistic reversal learning task). Overall, these studies demonstrate that acute PPX treatment can alter reward-related decision making, regardless of the brain state of the individual. Thus, it appears that PPX alters decision making, particularly when probabilistic tasks are involved.

Recently, behavioral investigations into the differences between PD patients with ICDs (PD-ICD) on and off their medication have been made. Two studies by Voon and colleagues demonstrate that PD-ICD individuals display aspects of impulsivity, particularly on their medication. First, PD-ICD individuals have a bias towards riskier choices (Voon et al., 2011a), when they are on or taken off their medication (Voon et al., 2010). Second, in a temporal discounting task, PD-ICD individuals on 1mg PPX show a preference for smaller immediate rewards over larger delayed rewards, indicative of increased impulsive choice (Voon et al., 2011b). Another group replicated these findings, showing that PD-ICD individuals on their medication demonstrate higher

impulsive choice in a temporal discounting task as opposed to when they were off their medication (Housden et al., 2010).

Advances in the neurobiology of DA agonist-induced pathological gambling in PD patients have been made. Studies show that PD-ICD individuals process reward and risk differently from both PD patients without ICDS and healthy controls. Indeed, in the PD-ICD patients, there appears to be a reduction in activity in the frontostriatal circuitry that mediates self-control and an enhancement or sensitization of the mesolimbic system that mediates motivation. Several imaging studies have confirmed a decreased activity in the OFC and cingulate cortex in PD patients with DA agonist-induced pathological gambling. For example, in a PET study using a probabilistic feedback task, a DA agonist challenge in controls (PD patients with no ICD) increases activity in the OFC and cingulate cortex, whereas PD-ICD patients show decreases in activity of these brain regions (van Eimeren et al., 2010). In an fMRI study using a probabilistic feedback task, PD-ICD individuals made more riskier choices and demonstrated a reduction in activity in the OFC and ACC compared to PD patients without ICDs (Voon et al., 2011a). On the other hand, Steeves and colleagues reported an increase striatal DA release in PD-ICD individuals on vs. off medication during a gambling task (Steeves et al., 2009). At rest, imaging studies show that PD-ICD patients compared to PD patients without ICDs have greater activity in the hippocampus, AMG and VP (Cilia et al., 2008). These findings dovetail with the proposed circuitry that drives impulsivity (see Fig. 3).

Effects of pramipexole in rodent models of impulsivity and measurements of reward

To date, only a few preclinical studies have assessed the effects of PPX on impulsive decision-making. These studies focused on aspects of risk-taking as measured with probability discounting tasks or on impulsive choice, measure by temporal discounting tasks. For example, Fowler and colleagues studied the effects of acute PPX in two different rodent models of impulsivity. They found that PPX (0.1-0.3mg/kg) increases preference for gambling-like schedules of food reinforcement such that rats switch their preference from a fixed ratio-1 to a variable ratio schedule of reinforcement (Johnson et al., 2011). They also found that PPX influences impulsive choice such that rats prefer a smaller-sooner over a larger-delayed food reward when treated with 0.1 and 0.3mg/kg PPX; however, non-significant trends were found with 0.3mg/kg in a delayed discounting task that measures choice over a range of delays that increase throughout the session (Madden et al., 2010). Another lab reported an apparent increase in impulsive choice with PPX (0.32mg/kg) although there was a general decrease in choice of the large reinforcer independent of delay (Koffarnus et al., 2011). Collectively, these studies provide evidence that acute PPX influences several aspects of decision-making that contribute to impulsive behavior.

There are only two studies that have investigated the intrinsic rewarding properties of PPX and both investigated the influence of a PD-like brain state on the measured outcomes. The Napier lab demonstrated that PPX is sufficiently reinforcing to support acquisition of a place preference (Riddle et al., 2012) and revealed that a higher dose of (±)PPX (4mg/kg, ip) is necessary to induce a place preference in controls compared to PD-like rats in which 2mg/kg is sufficient. Another laboratory

demonstrated that rats self-administer PPX (0.25mg/kg/infusion), albeit at lower rates than other drugs of abuse such as cocaine (Engeln et al., 2012). Using a progressive ratio-3 task, they revealed weak motivational properties of PPX (i.e., mean break points are approximately 3-5 lever presses but range from 5 to 22 lever presses), while an extinction paradigm demonstrated that PPX does not sustain high seeking behavior (i.e., extinction occurs within 10 minutes). In all three experimental measures PD-like rats performance did not differ from sham controls. Overall, these two studies provide evidence that PPX has intrinsic rewarding properties, but further studies are needed to verify if a PD-like brain state modifies the behavioral outcomes.

Similar to other rewards, PPX can also enhance the motivational salience of reward associated cues. Woods and colleagues demonstrated that PPX enhances the reinforcing effects of cues that were previously paired with cocaine (Collins et al., 2011a). In the presence of the previously cocaine-paired cues, nose poking rates significantly increase during this substitution. Similar results were seen in a progressive ratio task. Moreover, rats pretreated with PPX demonstrated a significantly higher break point when they poke for the presentation of previously paired cocaine cues. Collectively, the studies described in this section indicate that PPX alters aspects of impulsivity, is intrinsically reinforcing, and can motivate reward-seeking behavior.

Involvement of the D2R family in impulsivity

Various impulsivity traits are linked to the D2-like receptor family. Genetic studies have associated impulsivity traits with both the D2R and D3R gene (Blum et al., 1995; Colzato et al., 2010; Forbes et al., 2009; Hamidovic et al., 2009; Noble, 2000;

Comings et al., 1994). Variants of the DRD3 gene are associated with ICD in PD (Lee et al., 2009b).

Several reports in both humans and animals have implicated low D2/D3R availability as factor underlying aspects of impulsivity. Volkow and colleagues used PET imaging in humans and demonstrated that cocaine and methamphetamine abusers, individuals that are considered to be impulsive, have lower D2R availability compared to controls (Volkow et al., 2001;Volkow et al., 1993). In rats, Dalley et al., (2007) found that accumbal D2/D3R availability negatively correlated with impulsivity. Moreover, impulsivity, as measured in a five-choice serial reaction time (5-CSRT) is directly correlated with higher rates of cocaine self-administration (Dalley et al., 2007). Several other studies correlate various measures of impulsivity with low D2/D3R availability in the striatum (Reeves et al., 2012; Ghahremani et al., 2012; Lee et al., 2009a). Low expression levels of midbrain D2/D3 autoreceptors are also associated with aspects of impulsivity in humans and monkeys (Zald et al., 2008; Nader et al., 2006; Buckholtz et al., 2010). For example, Nader et al. (2006) used PET to measure D2R availability in the basal ganglia. They demonstrated in monkeys that D2R availability (before drug intake) is inversely related with rates of cocaine self-administration. Furthermore, cocaine intake produces a robust decrease in D2R availability. During abstinence from cocaine, D2R availability recovers in some monkeys. In humans, trait impulsivity is negatively correlated with availability of D2/D3R (Buckholtz et al., 2010). This study also revealed that autoreceptor availability is negatively correlated with the magnitude of amphetamine-induced DA release in the striatum that predicted stronger subjective "wanting" for more drug following the amphetamine treatment. These findings are in

line with the theory of incentive salience which predicts that hyperactivity of DA striatal release enhances motivational salience (Berridge and Robinson, 1998), a driving force in impulsive behaviors.

However, there are conflicting reports regarding the expression of D3Rs during conditions of sustained increases in dopaminergic transmission (i.e., repeated treatments with DA agonists or in individuals that chronically abuse drugs). Studies showed that there is actually an upregulation of D3Rs and, when concomitantly measured a downregulation of D2Rs. For example, in human poly drug users that include methamphetamine a PET study showed an upregulation of D3Rs in the SN and VP (Boileau et al., 2012b). Animal studies support these findings. In rats, a 14 day treatment with D2/D3 agonists (7-OH-DPAT or quinpirole) increases expression of D3Rs in the VP and SN, and decreases D2R in the VP, SN, and NA (Stanwood et al., 2000b). Fourteen days of PPX treatment also leads to increases in NA D3Rs (Maj et al., 2000; Tokunaga et al., 2012). Moreover, rats that demonstrate cocaine-induced locomotor sensitization have an increase in D3R density and a decrease in D2R density in the ventral striatum 42 days after the last cocaine treatment (Collins et al., 2011b).

A positive correlation between D3Rs and impulsivity makes sense considering the findings over the past decade that antagonism of D3Rs attenuates actions of several abused drugs in various rodent models of drug addiction (for review, see (Heidbreder and Newman, 2010). Collectively, studies indicate that D3Rs are not involved with the rewarding effects of drugs and sucrose *per se*, but rather the motivation to obtain and/or to seek them out. For example, D3R antagonists do not affect responding on low fixed ratio (FR-2) conditions but do decrease responding with higher fixed ratios and

progressive ratio tasks, which are considered to require more effort in order to obtain the reward (Higley et al., 2011; Gilbert et al., 2005; Vorel et al., 2002). Regarding impulsivity, 100µg/kg PPX, a dose that is thought to be D3R-selective, and 300µg/kg PPX, a dose thought to be more D2/D3R selective (Collins et al., 2009; Collins et al., 2007; Collins et al., 2005), alter gambling-like behaviors (Johnson et al., 2011) and delayed discounting (Madden et al., 2010) in rats. This suggests that D3Rs as well as D2Rs are driving PPX-induced impulsivity. A recent neuroimaging study supports the concept that D3Rs have a role in reward-related behaviors; pathological gamblers have a positive correlation between D3R levels and gambling severity and impulsiveness (Boileau et al., 2012a).

Regulation of D2R and D3Rs in Parkinson's disease

The regulation of D2/D3R in PD may play a role in expression of impulsive behavior. Dopaminergic cell loss in PD and denervation of output structures leads to an *increase* in D2Rs and a *decrease* in D3Rs (Rinne et al., 1990; Brooks et al., 1992; Ryoo et al., 1998). For example, human PET studies reveal an upregulation of striatal D2R in non-treated PD patients (Rinne et al., 1990). Striatal D2R adaptations have been contributed to a denervation supersensitivity, in which the system compensates for the decreased levels of DA (Lee et al., 1978). However, such compensation does not occur with the D3R. Recent studies show that drug naïve patients with early stage PD have a decrease in D3R expression in the ventral striatum and GP (Boileau et al., 2009). Similar changes in D2/D3Rs have also been found in animal models of PD (Graham et al., 1990). In rats with a unilateral 6-OHDA-induced lesion to the MFB, there is a decrease in D3R

expression and mRNA in the nucleus accumbens (NA) and SN ipsilateral to the MFB lesion (Bordet et al., 1997; Stanwood et al., 2000a; Levesque et al., 1995). D3R loss is consistent with loss of dopaminergic terminals. In contrast, D2R expression and mRNA are increased (Levesque, 1995; Stanwood et al., 2000a). Moreover, D3R mRNA is not altered in the dorsal striatum following MFB 6-OHDA-induced lesions (receptor levels were not measured; Bordet et al., 1997). D3R adaptations are also linked to brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor related family of neurotrophins. BDNF is found in many brain regions, including the VTA (Seroogy et al., 1994) and SNpc (Hyman et al., 1991). BDNF, which can be transported anterogradely (Altar et al., 1997) and released upon depolarization (Thoenen, 1995) can control D3R expression (Guillin et al., 2001). Studies show D3R binding and mRNA levels in the NA are decreased after 6-OHDA-induced lesions of the MFB and impairments of fast anterograde axonal transport of midbrain dopaminergic neurons (Levesque et al., 1995). Based on these studies, it believed that destruction of the ascending dopaminergic neurons reduces release of BDNF onto terminal regions that consequently leads to a decrease in D3Rs expression.

Whether these region specific alterations in D2R upregulation and D3R downregulation influence impulsive behavior in PD patients is unknown, but these receptors profiles suggests a decrease in impulsive behavior, which has generally been described in PD patients (Menza et al., 1993; Tomer and Aharon-Peretz, 2004; Bodi et al., 2009). Moreover chronic treatment with DA agonists produce the opposite receptor adaptations as seen with DA deafferentation. For example, in rats a fourteen day treatment with D2/D3 agonists, 7-OH-DPAT or quinipirole, increases expression of

D3Rs in the VP and SN and decreases D2R in the VP, SN and NA (Stanwood et al., 2000b). Rats treated for 14 days with PPX (0.3 and 1mg/kg) show increase expression of D3Rs in the NA (D2R expression was not studied; Maj et al., 2000; Tokunaga et al., 2012). Thus, given the important roles of D2/D3Rs in impulsivity it is interesting to speculate that the dysregulation of D2/D3Rs in a PD-like brain state as well as following chronic treatment with DA agonists may play a factor in susceptibility to PPX-induced ICDs.

Significance

The goal of this thesis dissertation is to expand our knowledge on the neuropsychopharmacology of PPX-induced impulsivity. From this literature review, it can be seen that characterization of the neurobiology of PPX-induced ICDs is ongoing. Progress in elucidating the effects of acute and chronic PPX has been in made in the clinic. Slower progress has been made regarding animal models that study PPX-induced impulsivity. These few studies have only looked at behavioral effects with acute PPX. We sought to determine if PPX would alter risk-taking, one aspect of impulsivity, in a rodent model of PD as well as in controls using our novel ICSS-mediated probability discounting paradigm. Our findings add to the literature as we have included assessments of both PD-like rats and chronic treatment with withdrawal and subsequent reinstatement of PPX treatment. The literature supports a role of the VP in reward-motivated behaviors, particularly with salience attribution. This brain region is embedded within the limbic-motor circuitry where PPX is known to influence the neuronal activity. Moreover, D3Rs, which are implicated in reward-seeking behaviors, are expressed in

both the VP and the related limbic circuitry. The second focus of this dissertation was to determine if PPX, given systemically at doses known to alter aspects of impulsivity in the rat, could alter neuronal activity in the VP. Further, we assessed the ability of a D3R antagonist to alter these effects. The literature provides us with ample evidence that D3R expression is regulated by dopaminergic tone. Thus, we also assessed the effect of systemic PPX on neuronal firing in both rats treated chronically with PPX as well as in two different rodent models of PD. Our findings revealed that the VP is engaged by a range of PPX doses known to alter reward-related behavior. These changes in VP neuronal activity are under the influence of D3Rs. Our data provide evidence that rats treated chronically with PPX enhanced the potency of PPX to alter VP neuronal firing rate. A late, but not early, stage model of PD, showed a trend to enhance the potency of PPX. These findings will help guide the field of behavioral addictions to determine the neuroanatomical substrates and receptor subtypes involved in PPX-induced effects on reward-related behaviors.

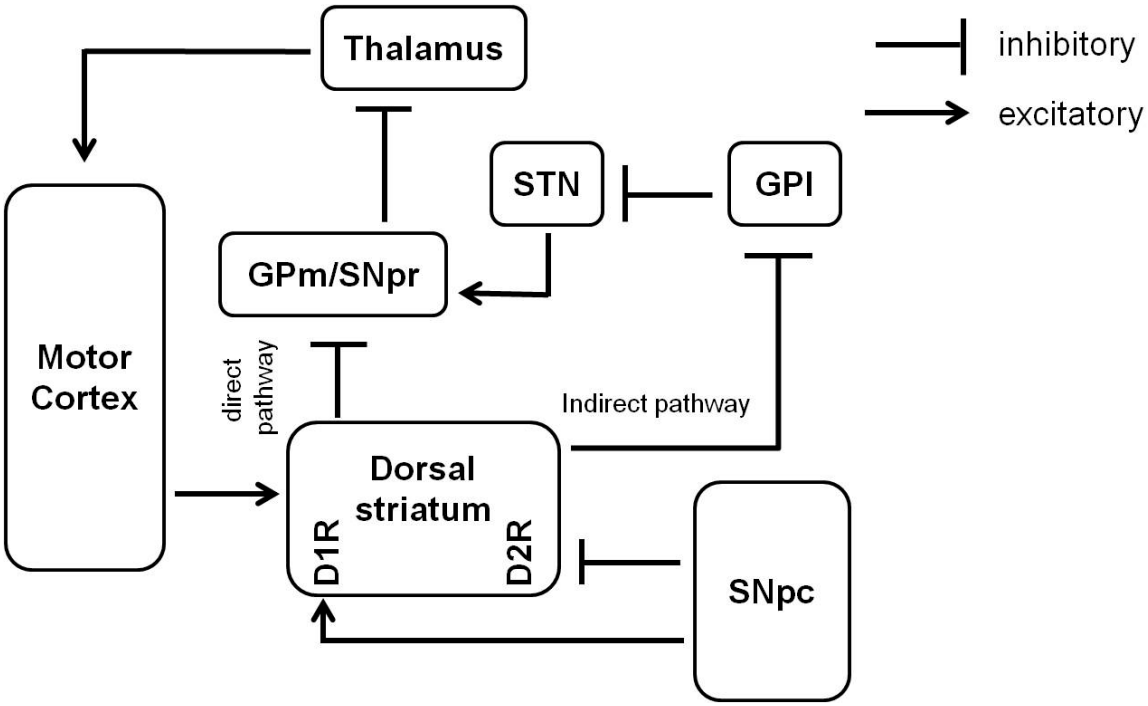


Figure 1. Schematic of the "backbone" of basal ganglia circuitry based on output pathways from the dorsal striatum. The direct pathway provides a direct connection from the dorsal striatum to GPm/SNpr. The indirect pathway is comprised of the dorsal striatum-GPl-STN-GPm/SNpr connections. Enclosed areas represent the following brain structures: GPl, lateral globus pallidus; GPm, medial globus pallidus; SNpr, substania nigra pars reticulata; SNpc, substantia nigra pars compacta; STN, subthalamic nucleus. Note: the projection from the SNpc is largely dopamine, and the illustrated excitatory *vs* inhibitory influences on the dorsal striatum are lent by the activating striatal D1R versus D2R, respectively.

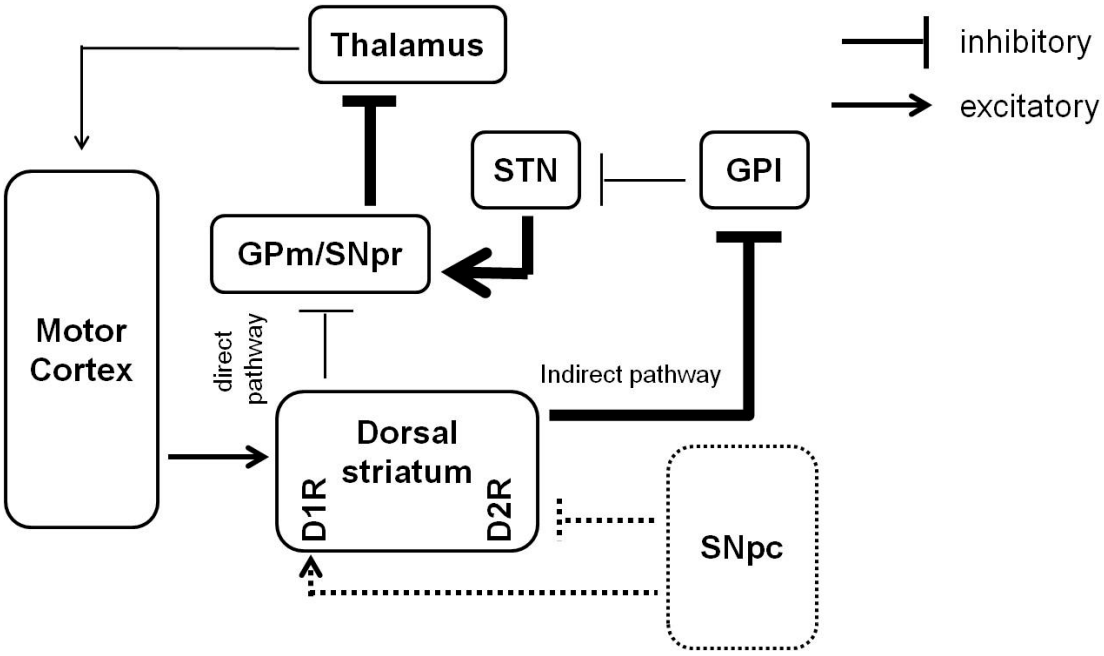


Figure 2. Schematic of the changes proposed to occur in the basal ganglia circuitry following degeneration of the nigrostriatal pathway (i.e., a parkinsonian brain state). Dotted lines indicate degeneration of the brain region and its projections. Compared to Fig. 1, the increase in width of a line indicates a larger influence from the originating brain structure. A decrease in width of the line indicates a smaller influence from the originating brain structure. Enclosed areas represent the following brain structures: GPl, lateral globus pallidus; GPm, medial globus pallidus; SNpr, substania nigra pars reticulata; SNpc, substantia nigra pars compacta; STN, subthalamic nucleus.

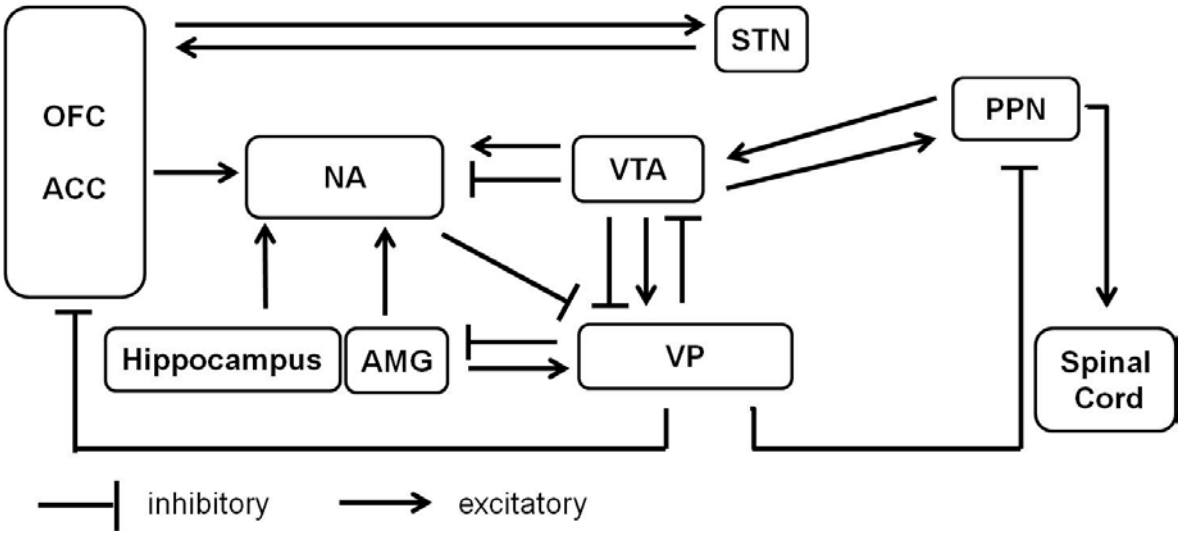


Figure 3. Schematic diagram of brain circuits that are likely involved in impulsivity, particularly reward-motivated motor behaviors that reflect risk-taking. Enclosed areas represent the following brain structures: ACC, anterior cingulate cortex; AMG, amygdala; NA, nucleus accumbens; OFC, orbitofrontal cortex; PPN, pedunculopontine nucleus; STN, subthalamic nucleus; VP, ventral pallidum; VTA, ventral tegmental area.

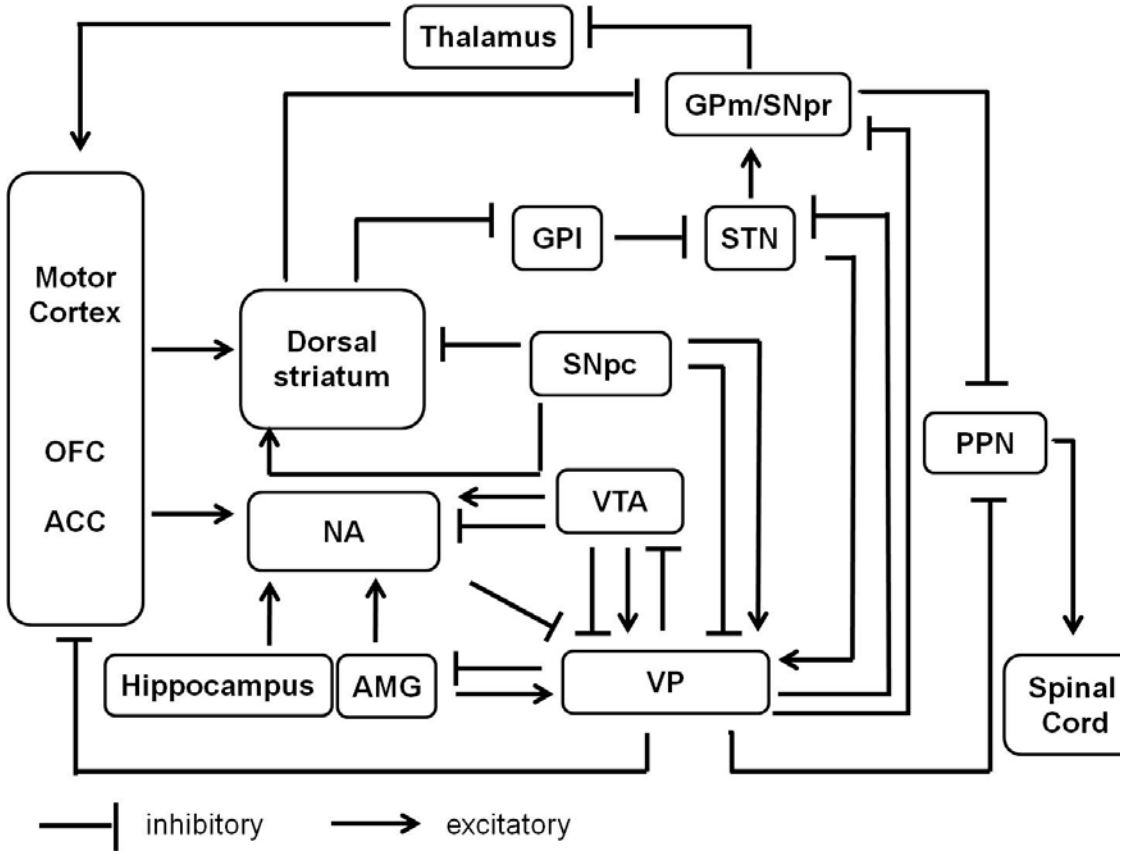


Figure 4. Schematic of the overlapping neurocircuitry between reward and motor systems. Particular focus for this dissertation is the overlapping connections of motor and reward systems that involved the VP. Enclosed areas represent the following brain structures: AMG, amygdala; NA, nucleus accumbens; GPl, lateral globus pallidus; GPm, medial globus pallidus; PPN, pedunculopontine nucleus; SNpr, substania nigra pars reticulata; SNpc, substantia nigra pars compacta; STN, subthalamic nucleus; VP, ventral pallidum; VTA, ventral tegmental area.

CHAPTER III

INTRACRANIAL SELF-STIMULATION AS A POSITIVE REINFORCER TO STUDY IMPULSIVITY IN A PROBABILITY DISCOUNTING PARADIGM

Abstract

Probability discounting is used to study risky decision-making in humans and rodents. In these paradigms, the subject chooses between a small reward that is always delivered and a large reward that is delivered with varying probabilities. Risk-taking is defined as a preference for the large, uncertain reward. The aversive consequence associated with this task involves choosing the large reward and not obtaining it. To study this form of impulsivity in rodents, food reinforcement is commonly used. Using this reinforcer, and the need to food-deprive rodents to enhance task performance, may be problematic in rodent models that exhibit eating disorders, in pharmacological assessments that alter feeding, and for assessments of the neurocircuitry that is engaged by both feeding and risk-taking. We reveal here that electrical intracranial self-stimulation (ICSS) can be used as the positive reinforcer in risk assessments (i.e., probability discounting). ICSS was selected as it is rapidly acquired, the operant procedures are retained for months, and no tolerance or satiety develops to the reinforcer; thus, ICSS can be used in multiple test sessions in a repeated measures design. We developed an efficient, standardized, six phase ICSS-mediated protocol that allowed for assessments of risk-taking in a probability discounting task. We demonstrated that the

discounting behavior remained stable for several weeks. The value of this protocol is discussed in terms of practical as well as theoretical advantages of using ICSS-mediated reinforcement.

Introduction

Impulsivity can be regarded as "actions that appear poorly conceived, prematurely expressed, unduly risky, or inappropriate to the situation" (Daruna and Barnes, 1993). While some beneficial aspects of impulsivity are known (Dickman, 1990), it is generally recognized as a dysfunctional trait that is frequently associated with numerous neurological and psychiatric disorders including frontal lobe damage, schizophrenia, attention deficit-hyperactive disorder and substance abuse disorders. According to the American Psychiatric Association, impulse control disorders (ICDs) are a form of psychiatric disorder (American Psychiatric Association, 2000). ICDs include trichotillomania, intermittent explosive disorder, pathological gambling, kleptomania, pyromania, hypersexuality, compulsive shopping and others.

To understand impulsivity and ICDs, and to subsequently develop therapies targeted to particular aspects of the disorder, laboratory protocols that model attributes of impulsivity are required. Risky decision-making is one facet of impulsivity. A common method used to study risky choice in both humans and laboratory rodents is the probability discounting paradigm (Mobini et al., 2000; Rachlin et al., 1991; Richards et al., 1999). In this task, the subject can choose between a small reward that is always delivered and a large reward that is delivered with varying probabilities. Risky behavior is defined as a preference for the large uncertain reward. The aversive consequence

associated with this task involves choosing the large reward and not obtaining it (Cardinal and Howes, 2005). In rodent testing of probability discounting, food is often used as the positive reinforcer and to motivate the animal, salience of the food is enhanced by food-deprivation. This approach presents several disadvantages which can potentially confound outcomes. First, internal factors, such as hunger or thirst, can themselves lead to a change in impulsive behavior in animals (Minamimoto et al., 2009; Schuck-Paim et al., 2004). Second, chronic food restriction can lead to adaptations in dopaminergic (Carlson et al., 1988; Carr et al., 2009; Carr et al., 2003; Collins et al., 2008) and serotonergic signaling (Haleem and Haider, 1996; Huether et al., 1997; Kohsaka et al., 1980). These neurotransmitters also play a role in impulsivity (Adriani et al., 2009; Mehlman et al., 1994; Mobini et al., 2000; Soubrié, 1986; Winstanley et al., 2005). Moreover, this reward option may not be possible for assessments of risky choice in rat models of human neuropathologies that present eating disorders or for testing pharmacologics that alter feeding behaviors. Thus, we sought to design a probability discounting paradigm that utilized a positive reinforcer that avoided such shortcomings. To be broadly applicable to a range of laboratory assessments, we determined that criteria for this reinforcer should include the following: **(i)** it should more directly engage brain "reward centers" than is possible with food reward. **(ii)** It should be conducive to robust operant task testing. **(iii)** It should demonstrate a range of reward values that can be discriminated by the rat. Finally, **(iv)** it should support stable responding for several weeks. We reveal here that ICSS meets these criteria. In ICSS procedures, rats perform an operant task to obtain a positive reinforcing current delivered *via* an electrode implanted in reward regions of the brain (Olds and Milner, 1954). For the current study,

we selected the medial forebrain bundle (MFB) at the level of the lateral hypothalamus (LH) as the stimulation target. This structure is well known to readily support ICSS with a large range of stimulation parameters. We detail how this reward parameter can be successfully implemented for probability discounting paradigms, and we verify performance stability and persistence.

Methods

Male Sprague-Dawley rats weighing 250-274g upon arrival (Harlan, Indianapolis, IN) were housed in pairs under environmentally controlled conditions (7:00AM/7:00PM light/dark cycle, temperature maintained at 23-25°C) with access to rat chow and water *ad libitum*. All rats were handled according to established procedures in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, Washington DC); specific protocols were approved by the Institutional Animal Care and Use Committee at Rush University Medical Center.

Implantation of electrode into the lateral hypothalamus

Eight rats were anesthetized with sodium pentobarbital (50mg/kg ip; Sigma, St. Louis, MO), and placed into a small animal stereotaxic instrument (David Koft, Tujunga, CA) with the nose piece set at 3.3mm below the horizontal. A midline scalp incision was made and a hole was drilled through the skull at -2.8mm posterior to bregma and 1.8mm lateral to midline. A bipolar stimulating electrode (MS303/3-B/SPC; Plastics One, Roanoak, VA) was stereotaxically lowered -8.4mm from dura into the LH. Electrodes were secured to the skull with stainless steel screws and dental acrylic, and the incision

was sutured. Rats were returned to their home cage following full recovery from anesthesia, and one week later, testing in the operant chambers was initiated.

Test Apparatus

Rats were tested in operant chambers (30.5 cm x 24.1cm x 21.0 cm; Med-Associates, St. Albans, VT), enclosed in ventilated, sound attenuated boxes outfitted on one wall with two retractable levers and a stimulus light above each lever. On the opposite wall, a single 100mA house light was located in the top center. Intracranial stimulation was delivered by constant current stimulators (PHM-152/2 Dual programmable ICSS stimulator) *via* bipolar leads connected to 2-channel commutators (Plastics One, Roanoak, VA) mounted above the chamber.

Behavioral testing protocol

Acquisition of the probability discounting task was accomplished with a six phase protocol. Each phase included ongoing assessments of individual task performance, and the protocol was designed to sequentially fine-tune and verify the ICSS parameters as the rats progressed through the phases in order acquire the probability discounting task. As rats advanced, they were trained to build on prior task performance in order to meet standardized phase milestones. Table 1 illustrates the time-line for the protocol, as well as the objectives, criteria and maximal number of sessions necessary for rats to complete phase criteria. The methodologies associated with each phase, along with an explanation of data analyses, are provided below.

Phase 1: Shaping

Following one week recovery from surgery, rats were trained to lever press to obtain a positively reinforcing electrical brain stimulation (EBS) using shaping procedures modified from Chester and colleagues (Chester et al., 2006). At the beginning of a 30min session, one of the two levers was extended. Shaping occurred by successive approximation, during which experimenter-applied EBS was used to initially direct the rat towards the lever, and then to aid the rat in making the association between a lever press and receiving an EBS. At the start of this process, each EBS consisted of biphasic 100μA square wave pulses applied as a 100Hz for 500μs. Each pulse was 200μs long and a 100μs delay separated each pulse. With the EBS frequency and pulse duration remaining constant, the current intensity was adjusted for individual rats based on their performance to approach and ultimately press the lever. The procedure used for this adjustment was as follows: Lack of behavioral responses (e.g., sniffing, rearing, approaching the lever) resulted in 20μA increasing increments. If responses indicative of aversion occurred (freezing, crouching, twitching) the current was decreased by 20μA increments until aversive behaviors were no longer observed. Once the rat pressed the extended lever eight times in approximately one min, that lever was retracted and the other lever was extended and shaping proceeded (the order of left *vs*. right lever presentation was counter balanced across sessions). The minimum criteria set for this phase was steady lever pressing (~eight presses/min) on both levers. Once lever pressing was established, the current intensity was incremented until no further increase in lever pressing rate was seen. This intensity level was used for the remaining Phases.

Phase 2: Training on Fixed Ratio (FR)-1

The purpose of this Phase was to demonstrate stable lever pressing rates. To do so, one lever was extended during each 30min session (right and left levers were counter balanced across sessions) and the number of lever presses was recorded. To complete this phase, rats had to meet the following minimum criteria in consecutive sessions: **(1)** lever press at least five times within the first two min of the session (i.e., to initiate the session), and **(2)** display a minimum average of eight lever presses per min in the session. Lever pressing rates for the last two sessions were averaged for each rat and group means ± SEM are reported. Data were analyzed using a paired *t*-test with significance set at $p<0.05$.

Phase 3: Rate-Current Intensity Functions

The purpose of this Phase was to determine the effect of various LH stimulation parameters on the rate of lever pressing. A single lever was used in a session which was approximately 30-40min in duration (right and left levers were presented in a counter balanced order across sessions). To evaluate the impact of various current intensities on lever press response rate, *rate-intensity functions* were generated for each rat. In this task, the LH stimulation frequency (100Hz) and train duration (500μs) where held constant while various intensities were pseudo-randomly presented. In the first 30sec of the session, rats had access to the lever which was set to deliver the current intensity used to meet Phase 2 criteria. This was used as a protocol 'reminder'; these data were not analyzed. Seventeen current intensity levels between 10 and 350μA were evaluated, and each of these levels was tested during a two min time period during which the lever was

extended, and number of lever presses was recorded. Following the 2min period, the lever was retracted for a 10sec time-out. The higher currents that produced maximal lever pressing responding were not sufficient to induce classical aversive-like behaviors. A curve comparing current intensity and number of presses was generated from each session and both the maximal number of lever presses (E_{max}) and the minimal number of lever presses (threshold) were determined using a non-linear regression (GraphPad Prism, La Jolla, CA). A third order polynomial was fitted to visualize these two features. To complete this phase, both the E_{max} and threshold had to be stable (i.e., exhibit < 20% variability) for three consecutive sessions. A final 'stable curve' was generated by averaging the three curves which met criteria and the currents that produced 90%, 60% and 40% of E_{max} were determined (i.e., effective current (EC); $ECur_{90}$, $ECur_{60}$ and $ECur_{40}$, respectively; GraphPad Prism 5.0). A linear correlation was conducted between lever pressing rate and EBS current intensity to verify that changes in EBS frequency altered ICSS.

Phase 4: Training in Discrete Trials

This Phase was designed to train rats to recognize the temporal nature of 15sec trials using each rat's own $ECur_{60}$ as the reinforcer. To do so, rats were trained on a simplified version of the full discounting task, as modified from Cardinal and Howes (2005). Each session consisted of 200 trials. The session began with both levers retracted and chamber lights off. Trials occurred at 15sec intervals. Two sec after the start of a trial, the house light was illuminated, followed three sec later by the extension of one lever. The rat had 10sec to press that lever one time, if the response was not

executed, the trial was aborted (termed an *omitted trial*), the lever retracted and the house light turned off. If a lever press was made, an EBS was delivered (i.e., ICSS occurred) and the stimulus light over the lever was turned on. After 0.5sec, all lights were turned off and the lever retracted. Levers were alternately extended among trials. The minimum criteria set for this phase was for rats to have 50 or less omitted trials per session for four consecutive sessions.

Phase 5: Choice Tests

The purpose of this Phase was to determine for each rat, a small and large reinforcer that could be used in the probability discounting phase. The Phase was designed to train rats to recognize and select, lever-specific, reinforcement values using the FR-1 discrete trials employed in Phase 4. The initial reinforcement values used were the current intensities that corresponded to the $ECur_{90}$ (i.e., large reinforcer) and $ECur_{40}$ (i.e., small reinforcer) obtained for each rat in rate-intensity functions in Phase 3. Each session consisted of three blocks. A block consisted of 20 forced-choice trials followed by 20 free-choice trials. In forced-choice trials, each lever was extended independently allowing the rat to associate particular reinforcement values with each lever. In free-choice trials, both levers were extended, giving the rat an opportunity to choose between levers and thus demonstrating reinforcement preference. Across the three blocks, the reinforcement value associated with the left lever changed from no EBS (i.e., "no reinforcer"), to $ECur_{90}$, to $ECur_{40}$. The reinforcement value associated with the right lever changed from $ECur_{40,}$ to no EBS, to $ECur_{90}$. The lever associated with each sequence of reinforcement values was counter balanced across sessions. To determine a

reinforcement preference in each block, data were analyzed from free-choice trials. Reinforcement preference was defined by the "*free-choice ratio*"; i.e., the number of selections for the large reward divided by the total number of lever responses made x100. The first two blocks allowed the rat to demonstrate that the given EBS was reinforcing, (as indicated by the continued selection of that lever over the lever that offered no EBS). The third block provided a means to verify that the rats could *distinguish* between the various reinforcement values. As rats innately prefer a large reinforcer over a small reinforcer, lever selection over the 20 free-choice trials provided an index of that preference.

To complete this Phase, rats had to choose the larger of the two reinforcers in each block, on average, at least 70% of the time for three consecutive sessions. If a rat failed to achieve this criterion in any of the blocks for two consecutive sessions, the larger reinforcer value was adjusted by increasing the current intensity in 5μA intervals until the rat met the 70% criteria across blocks (without demonstrating behaviors indicative of aversion, e.g., freezing). It was required that reinforcer values remained the same for the final three test sessions in which the minimum criteria were met.

Phase 6: Probability Discounting Task

The purpose of this Phase was to determine the relationship between a rat's selection of a large reinforcer and the probability of that reinforcer being delivered. Procedures were modified from Cardinal and Howes (2005). The modifications involved reducing the time needed for each of the trials (as ICSS occurs at a quicker pace than self-administration of food). This allowed us to increase the number of trials and blocks

in each session; therefore, each session consisted of six or seven blocks. Each block consisted of 20 forced-choice trials followed by 20 free-choice trials. Trials occurred in consecutive 15sec intervals. For each session, one lever was designated the "small reinforcer/certain" (SC) lever and the other was the "large reinforcer/risky" (LR) lever. Thus, selection of the SC lever *always* resulted in delivery of the small reinforcer, while selection of the LR lever resulted in delivery of the large reinforcer but with various probabilities. SC and LR lever designation was kept constant for each rat, but counterbalanced across rats. The values of the small and large reinforcers corresponded to those used at the end of the Choice Test phase for current intensity. The large reinforcer probabilities used were 1.0, 0.85, 0.65, 0.5, 0.25, 0.125 and 0.0625. As carried out by Cardinal and Howes (2005), these probabilities were systematically decreased across the blocks in each session. Rats underwent 14 baseline sessions, most often as twice daily for seven days. Occasionally, testing occurred only once a day. There was no difference between morning and afternoon curves, nor was there a difference between curves tested once or twice a day (data not shown). In cases when rats were tested twice a day, the two sessions were averaged and the mean was used to illustrate discounting for that day. If only one test session was run in a day, the single curve represented discounting for that day. The daily discounting curves for each rat were averaged across rats to yield a daily group mean ± SEM. Data from free-choice trials of each probability (i.e., each block) were analyzed to determine a free-choice ratio (i.e., number of selections of the LR lever divided by the total number of lever responses made x100) *vs.* probability function. Two aspects of the group behavior were determined, acquisition and stability. A linear correlation was conducted between free-choice ratio and

probability magnitude to determine if the group acquired the discounting task. A two-way repeated measures (rm)ANOVA with 'day' and 'probability' as the factors was conducted to determine stability. Stability was defined as a significant main effect ($p < 0.05$) for the probability factor, but no main effect of day or interaction ($P > 0.1$) across three consecutive daily discounting curves (St Onge and Floresco, 2009; Winstanley et al., 2003).

Persistence of Stable Probability Discounting Behavior

It was of interest to determine if the discounting behavior endured, and to ascertain the utility of the model for assessments of pharmacological interventions. To make these determinations, a subset of rats (n=3) underwent a "mock treatment protocol" consisting of twice daily injections of saline (0.9% NaCl; 0.1ml/kg, ip) for two weeks (corresponding to days 38 to 51 of the protocol). Probability discounting was evaluated on six days during this time period. On the test day, discounting was evaluated one and 6.5hr after the morning injection; the two sessions were averaged for each rat on each test day and a group mean ± SEM was determined for each day. The small and large reinforcers corresponded to the current intensity values used during baseline data collection. Subsequently, we desired to determine if stable discounting behavior could be maintained if testing occurred less frequently. Thus, at the end of the saline treatment, rats were tested four and eight days later (corresponding to days 55 and 59 of the protocol). Rats were tested twice on each test day, the daily sessions were averaged for each rat and a group mean ± SEM was determined for each day. To determine persistence and stability in the free-choice ratio, the daily discounting curve from the last

day of baseline was compared to both the daily discounting curve from the 14th day of saline treatment (day 51) and from day 59. Data were analyzed using a two-way rmANOVA with 'time' and 'probability' as factors. Stability was defined as described for Phase 6.

Frequency as the Reward Modality in a Probability Discounting Task

This experiment was performed to ascertain if probability discounting could be obtained if the small and large reinforcer values differed in levels of current stimulation *frequency* (Hz) , rather than stimulation intensity (i.e., µA). Rats (n=3) that had completed the saline treatment and persistence protocol above were retrained in Phases 3, 5 and 6 using Hz as the ICSS dependent variable. First, lever pressing rate *vs.* ICSS current frequency function (i.e., *rate-frequency function*) determined the impact of varying stimulation frequencies on lever press response rate. For this assessment, the train duration (500µs) and current intensity (set at $ECur_{60}$- $ECur_{90}$ as determined for each rat in the prior study) were held constant while 17 various frequencies levels ranging from 5-140Hz (pseudo-randomly presented) were evaluated for ICSS behavior. The resulting rate-frequency curves were analyzed as in Phase 3 for rate-intensity curves. Next, rats were assessed in the Choice Test (as described above for Phase 5) wherein their ability to recognize levers differing from reinforcement values based on stimulation frequency was determined. The initial reinforcement values used were the EBS frequencies that corresponded to the $ECur_{90}$ (i.e., large reinforcer) and $ECur_{40}$ (i.e., small reinforcer) obtained from each rat's rate-frequency curve. After the rats met the Choice Test criteria, they were tested in the Probability Discounting Task. The large reinforcer

probabilities used were 1.0, 0.85, 0.65, 0.5, 0.25, and 0.0625. Rats were tested twice a day for two consecutive days. For each rat, a daily discounting curve was determined and the group daily mean was obtained. The two group daily means were averaged to yield a mean± SEM.

Histology

At the conclusion of the behavioral assessments, all rats were deeply anaesthetized with chloral hydrate (400mg/mg; Sigma, St. Louis, MO), and killed by a transcardial perfusion. For some rats, this was accomplished using ice cold 0.9% NaCl followed by 4% paraformaldehyde solution. Other rats received a 5V current (DC) applied to the stimulating electrode for 30sec to deposit iron and/or produce a very discrete lesion at the electrode tip. The iron deposits were visualized by a blue coloration produced *via* trychloroacetic acid (0.5%) and potassium ferricyanide (3%) added to a 4% paraformaldehyde solution used for transcardial perfusion after perfusing with ice cold 0.9% NaCl. Brains were removed, post-fixed in either 4% paraformaldehyde or 10% formalin before being stored in a 30% sucrose solution. Brains were sliced in 40μm coronal sections, mounted on slides. For all rats, stimulation electrode tip placements (indicated by an iron-reactive dye or a tissue lesion) were confirmed by two independent observers.

Results

Phase 1: Shaping

For this Phase, rats had to learn the association between a lever press and

receiving an experimenter-applied EBS. Rats with stimulation electrode tips placed in the MFB (n=6; Fig. 5) met the minimum criteria of eight lever presses/min on both levers within three sessions (corresponding to protocol test days 7-8). Rats with electrode tips outside the MFB (n=2; Fig. 5), did not successfully shape after six sessions and subsequently were removed from the study. For the six rats with proper the tip location, there was no correlate with any ICSS current or behavior measurements which are described below.

Phase 2: Training on FR-1

For this Phase, rats had to demonstrate steady lever pressing on both the left and right lever. To pass this Phase, rats had to initiate lever pressing at the beginning of each session and maintain a minimum average of eight lever presses/min for four consecutive sessions. All six rats met these criteria within four sessions (corresponding to protocol test days 9-10). The average lever presses/min for the left and right lever was 25 ± 4 and 27 ± 4, respectively. The rate of responding on either lever was not different (paired *t*-test p= 0.13), suggesting no lever bias. The range of current intensities used was 100-260μA.

Phase 3: Rate-Intensity Functions

For this Phase, rate of lever pressing was evaluated as a function of various current intensities. All six rats met the minimum criteria (e.g., stable E_{max} and threshold for three consecutive sessions) within eleven sessions (corresponding to protocol test days 11-16). Based on the average of the three curves which met stability criteria, the

final E_{max} ranged from 66 to 157 lever presses per two min. The final threshold ranged from 5 to 12 lever presses per two min. Verifying that magnitude of EBS current intensity (i.e., μA level) incrementally altered ICSS lever pressing, the final stable curves for all six rats exhibited a significant linear regression with r^2 values ranging from 0.74 to 0.95, $p < 0.01$. A representative rate-intensity curve of an individual rat is shown in Fig. 6A.

Phase 4: Training in Discrete Trials

For this Phase, rats were trained on a simplified version of the probability discounting task. The minimum criteria were 150 or more successful trials per session (which consisted of 200 total trials) for at least four consecutive sessions. All six rats met these criteria within six sessions (corresponding to protocol test days 17-19). Throughout all sessions, the average number of omitted trials was 16 ± 3. The range of current intensities used for training was 122 to 263μA, with an average 203 ± 21μA.

Phase 5: Choice Tests

For this Phase, rats had to demonstrate reinforcement preference for a lever associated with a reinforcer from one without a reinforcer, as well as preference for a lever associated with a large reinforcer from a small reinforcer. The minimum criteria were to choose the larger of the two reinforcers in each block, on average, at least 70% of the time for three consecutive sessions. All six rats met these criteria within 14 sessions (corresponding to protocol test days 20-26). The starting range for the large reinforcer (i.e., $ECur_{90}$) was 167 to 364μA; the average was 278 ± 28μA. Adjustments were made for three of the six rats, and the new values corresponded to $ECur_{91}$ to $ECur_{92}$ so that the

final range for the large reward was 167 to 374μA, with an average of 281 ± 29μA. Intensities for the small reinforcer (i.e., $ECur_{40}$) ranged from 106 to 235μA, with an average of 177 ± 20μA. Reinforcement preference across the three blocks for sessions that met criteria were 91 ± 0.02%, 94 ± 0.02%, 90 ± 0.02%, respectively.

Phase 6: Acquisition and Stability of Probability Discounting

As a group, rats acquired the probability discounting task in the first test session (Fig. 7). A linear regression revealed a positive correlation (r^2 =0.70; $p<0.01$) between selection of the LR and probability magnitude. In other words, selection of the large reinforcer decreased as the probability for its delivery decreased. This process was quantified by the "free choice ratio' i.e., selection of the LR lever/total number of responses. As a group, rats achieved stable daily discounting by the fourth test day. Based on the free choice ratio obtained on the second, third and fourth day (taken on protocol days 28-30) a two way rmANOVA revealed no effect of 'day' $F_{2,15}=0.85$, $p=0.45$ an effect of 'probability' $F_{6,90}=69.25$, $p<0.01$ and no interaction $F_{12,90}=0.26$, $p=0.99$. To determine that discounting remained stable across longer periods of time, rats continued to be tested in the discounting task for an additional 4-7 days (protocol days 34-37). Discounting remained stable; based on the free choice ratio obtained on final three test days, a two way rmANOVA revealed no effect of 'day' $F_{2,15}=0.79$, $p=0.47$ an effect of 'probability' $F_{6,90}=104.80$, $p<0.01$ and no interaction $F_{12,90}=0.84$, $p=0.61$.

Persistence of Stable Probability Discounting Behavior

Fig. 8 shows that rats demonstrated persistent stability in their behavior following

14 days of saline treatment (protocol days 38-51) as compared to the last day of baseline discounting in Phase 6. Furthermore, when testing was separated by three non-testing days stability endured; that is, tests were conducted on protocol days 55 and 59, and no difference was obtained between these two tests. These observations were demonstrated using a two way rmANOVA which revealed no effect of 'day' $F_{2,6}=0.29$, $p=0.75$ (i.e., the last day of baseline i.e., protocol day 37, the last day of saline treatment, day 51, and the end of the study, day 59) an effect of 'probability' $F_{6,36}=92.55$, $p<0.01$ but no interaction $F_{12,36}=0.37$, $p=0.97$. Overall, rats maintain a profile that was not indicative of risky behavior. At the lowest probabilities, rats preferred the lever that always delivered a small reinforcer, thus avoiding the aversive consequence of pressing the risky lever and failing to obtain any reward.

Frequency as the Reward Modality in a Probability Discounting Paradigm

The above SC and LR lever selection results were based on small and large levels of ICSS current intensity, i.e., µA. To test whether this probability discounting paradigm can be used with a reward modality of stimulation frequency, i.e., Hz, three rats were assessed in a modified version of Phases 3, 5 and 6. The same set of minimum criteria described for the current intensity assessments was used for the current frequency assessments. For the lever pressing rate *vs.* current frequency stimulation (rate-frequency function) assessment, the train duration (500µs) and current intensity were held constant (the intensity ranged between 153 and 181µA for the three rats tested), while various frequencies ranging from 5-140Hz were evaluated for each rat. All rats met the minimum criteria within seven sessions. Based on the average of the three curves which

met stability criteria, E_{max} ranged from 60 to 81 lever presses per two min. The final response threshold ranged from 3 to 9 lever presses per two min. Shown in Fig. 6B is a representative rate-frequency curve of an individual rat. Verifying that increments in EBS current frequency altered ICSS lever pressing, the stable curves for all four rats exhibited a significant linear regression with r^2 values ranging from 0.84 to 0.87, $p < 0.01$. The range of $EFreq_{90}$ extrapolated from the stable curves was 113 to 152Hz; the average was 131 ± 11Hz. The range of $EFreq_{40}$ was 84 to 96Hz, with an average of 89 ± 4Hz.

In the Choice Test (Phase 5), the initial EBS values tested were $ECur_{90}$ and $ECur_{40}$. The large reinforcement value was optimized in two rats using by using $ECur_{93}$ to $ECur_{95}$. These corresponded to 123 to 162Hz, and an average of 138 ± 12Hz. Rats successfully met the minimum criteria for this Phase within eight sessions. As a group, the reinforcement preferences across the blocks for the last three sessions were 92 ± 0.03%, 100 ± 0.0%, 93 ± 0.02%.

For assessing probability discounting (Phase 6) using ICSS frequency as the reward modality, rats were tested twice a day for two consecutive days. Fig. 9 demonstrates that as the probability of receiving the large reinforcer decreased, there was a proportional decrease in the selection of the LR lever (i.e., free choice ratio; linear regression, $r^2 = 0.97$; $p < 0.01$). Comparing Fig. 9 with Fig. 8 reveals that rats displayed a similar discounting profile whether the choice in EBS reinforcer is based on or current intensity or frequency.

Discussion

The current report revealed that ICSS can be used as a positive reinforcer to study risk-taking in a probability discounting paradigm. With this paradigm, rats rapidly learned to lever press for EBS, and they reliably chose to receive a larger EBS more than a smaller EBS, regardless if the variable EBS modality was intensity or frequency, indicating the larger EBS was a stronger reinforcer. As the delivery probability of the large reinforcer decreased, rats decreased their selection for the lever associated with the large reinforcement (i.e. risky lever), and this behavioral profile remained stable for several weeks. These findings demonstrate that this paradigm can be used efficiently to assess risk-taking, including the effects that chronic manipulations (e.g., repeated pharmacological treatments) have on the behavior.

The described six phase protocol emulated several features of standard discounting paradigms that use food as the reinforcer (Cardinal and Howes, 2005). Rats learned the association between performing an operant task (e.g., lever pressing) and receiving a reinforcer (Phase 1) and were able to demonstrate stable and persistent lever pressing for the reinforcer (Phase 2). Training for the discounting task was accomplished with a simplified version of the full discounting task (Phase 4) and then the rats were moved to the full discounting task (Phase 6). Moreover, the behavioral profile generated from probability discounting with ICSS was similar to that obtained with food reinforcement (Cardinal and Howes, 2005); rats decreased their selection for risky lever as the probability of delivery for the large reward decreased.

In food reinforcement studies, it is typical to use one pellet as a small reinforcer and four (or more) pellets as the large reinforcer. This approach incorporates at least two

assumptions in well-trained rats: **(1)** Given a choice between the two, food-deprived rats will consistently select the larger number of pellets when both are offered at 100% probability (Ghods-Sharifi et al., 2009; St Onge and Floresco, 2009). **(2)** Food-deprived rats will consistently choose the single pellet when the larger number of pellets is delivered at a small probability (Cardinal and Howes, 2005). These assumptions are verified after discounting training and experimental procedures are performed, with the idea that the outcomes reflect selection behaviors exhibited throughout the prior testing sessions. ICSS procedures bypass the need to generalize reinforcer values, and specific current parameters for the small and large reinforcer can be easily identified for each rat. Two different procedures were used to individualize and fine-tune the final small and large reinforcer values to be used for the discounting task. First, the lever pressing rate *vs.* current intensity, or current frequency relationship was ascertained. This relationship is similar to a drug dose-response curve, where magnitude of the independent variable (dose, or in our case EBS current intensity or frequency) is correlated to the response, and with sufficient test range, the asymptotes that indicate threshold and E_{max} are determined. Based on the matching law, which states relative rates of response will match the relative 'rates of reinforcement [in] concurrent schedules of reinforcement' (Herrnstein, 1970), it was assumed that the more a rat lever pressed for a particular current intensity or frequency, the more reinforcing that particular current parameter was for the animal. Thus, current parameters that produced 90% maximal responding ($ECur_{90}$) and 40% maximal responding ($ECur_{40}$) were initially chosen to designate large and small reinforcers, respectively.

The Choice Test was the second procedure used to fine tune the small and large

reinforcement values. We verified that the current corresponding to $ECur_{90\text{-}95}$ and $ECur_{40}$ were reliably reinforcing, and that all rats choose the higher current value when the alternate lever presented a lower value or no EBS. Importantly, these were performed *before* training in the discounting task, thus, helping to assure reliability and salience of the reinforcement values during assessments of risk-taking. In a separate study, we have now verified that this discriminatory capacity is maintained during at least 19 days of probability discounting tests (Rokosik and Napier, 2012). These assessments verify that in spite of slight differences in electrode placement within the LH, and thus the potential for different neurons to be activated by the stimulation current (Fulton et al., 2006), the individualization of small and large reinforcers provided a means for the rats to robustly and stably identify difference in salience magnitude and to demonstrate reinforcement preference. In the Choice Test, the first two blocks included a situation where one lever delivered no EBS; therefore, there was a possibility that extinction learning may have occurred. However, the 'no EBS levers' were different for the first two blocks, and in the third block, both levers were associated with a reinforcer (albeit of different value). In spite of this, the rats consistently selected the larger reinforcer across all blocks, even if the larger reinforcer was previously associated with no EBS. Thus, if extinction learning did occur within a block, it did not alter the rats' ability to properly execute the Choice Test for subsequent blocks. This not only ensured the effects to extinction learning could be accounted for, but also verified that rats did not develop a chamber side/lever bias, and confirmed that reversal learning is intact (i.e., the ability to learn when previously reinforcing associations no longer apply and to change future actions accordingly). As deficits in reversal learning are associated with some forms of impulsivity (Berlin et al.,

2004) the ability to monitor this cognitive parameter is an additional valuable feature of the model.

The current study, in conjunction with a recent separate study (Rokosik and Napier 2012) demonstrates a number of advantages that discounting protocols with ICSS as the positive reinforce offer over food-reinforcement protocols: These include the following: **(1)** It is more efficient (in spite of the need to add two additional phases). For example, unlike food pellets where time is required to retrieve and consume the reinforcer, EBS is delivered and received immediately after the lever press. Consequently, trials can be shortened from the typical 30-40sec to 15sec. **(2)** Satiation, which is a concern in food reinforcement studies is not an issue in ICSS procedures (Olds, 1958) which affords the opportunity to increase the total number of trials and blocks (i.e., probabilities) per session. **(3)** The frequency of testing can be increased; the current study conducted two sessions/day. This allows for increased sample size, providing a better representation of the phenomenon and likely improving the ability to detect treatment differences. Efficiency was an objective of this paradigm, and twice daily testing allowed for a rapid assessment of stability and reproducibility. (**4)** The rats rapidly acquire the discounting task. The current study revealed that in stark contrast to studies using food reinforcement, a statistically significant positive correlation between probability of delivery of the large reinforcer and selection of the LR lever was obtained in the *first* test session. In food reinforcement studies (using either delay or probability discounting tasks) it can take approximately 10 testing sessions for this correlation to emerge (Evenden and Ryan, 1996; St Onge and Floresco, 2009). **(5)** Stability of the discounting behavior also develops rapidly. In the current study, rats achieved stable

behavior by the fourth test day. This is in stark contrast to food reinforcement studies were it can take approximately 30 days to reach stable criteria for the discounting task (Ghods-Sharifi et al., 2009; St Onge et al., 2010; St Onge and Floresco, 2009). **(6)** The probability order within a session can be readily manipulated. This is an important feature, as the order of presentation of the probabilities may affect subsequent lever selection. In the current study, a descending order of probabilities was used, as is common in food reinforcement studies (Cardinal and Howes, 2005). We observed that in well-trained rats, as the probability of delivery of the large reinforcer decreased from 1.0 to 0.0625, selection for the risky lever decreased from nearly 100% to 0%. This established that *predictable* near zero, probabilities resulted in a near zero response. In a separate study, we have tested the ability of rats to perform in the ICSS-mediated discounting task when the order of probabilities was pseudo-randomized (Rokosik and Napier 2012). These rats acquired the discounting task and met stability criteria in a similar time frame as rats in the current study. However, at the lowest probabilities, rats chose the risky lever a higher percentage of the time (i.e., between 30-50%). This indicates that when a given probability level did not predict subsequent probabilities, the motivation for the rat to continue to select the risky lever remained high, even when the odds of obtaining a large EBS were very low. Floresco and colleagues (St Onge et al., 2010) also attempted to use a mixed order of probabilities (i.e., 1.0, 0.125, 0.25 and 0.5) with food reinforcement. In contrast to our observations with ICSS, they reported that rats were not able to effectively perform the discounting task, for at the lowest probability (0.125), rats where still choosing the risky lever ~75% of the time, compared to 50% of the time when probabilities were presented in a descending systematic order. In

summary, while the predictability of the systematic decrease in probability appears to influence choice behavior in both food- and ICSS-mediated probability discounting, the robust and immediate nature of ICSS assisted the rats in adapting to the unpredictable changes. As probability discounting tasks are used as a tool to assess risky behavior, and unpredictability is a fundamental aspect of risk, the ability to randomize probability is another valuable feature of the current model. **(7)** The lever assignment of LR can be changed without interfering with task acquisition. Moreover, this method assures that lever selection does not reflect an innate, or non-reward-mediated, bias. In the current study, LR *vs.* SC lever assignment was counterbalanced between rats (but a consistent designation was used for each rat). In a separate study, we determined that similar discounting behavior was obtained when LR and SC were counterbalanced left *vs.* right among sessions (Rokosik and Napier, 2012). To our knowledge, such flexibility has not been demonstrated in discounting studies using food reinforcement. **(8)** ICSS bypasses a confound of dysregulation in energy balance, which can alter risk-taking. ICSS directly engages reward centers of the brain (Olds and Milner, 1954). This immensely salient modality is highly motivating so that the rats rapidly learn to associate EBS reinforcement in an operant task. The salience of food reinforcement is not robust and rats typically are food deprived to 80-90% of their free-feeding body weight to motivate them to learn and subsequently perform operant tasks (Weingarten, 1983). As rats progress through test sessions, there is concern that rats become satiated (Cardinal and Howes, 2005; St Onge and Floresco, 2009), and the energy balance of the animal can change. For example, risk-sensitive foraging theories, which state that if an animal is offered a choice between a fixed, predictable outcome *vs.* a variable, unpredictable

outcome, the decision will be based on the energy state of the animal (Stephens, 1981). Verifying these possibilities are reports demonstrating that alterations in hunger or thirst states, change impulsive behavior (Minamimoto, La et al., 2009;Schuck-Paim, Pompilio et al., 2004). **(9)** Finally, ICSS bypasses the possible influence that satiety state may have on the neurochemistry within brain networks involved in impulsivity. The neurotransmitters, serotonin and dopamine play a role in impulsivity in both humans and rodents (Adriani et al., 2009; Mehlman et al., 1994; Mobini, et al., 2000; Soubrié, 1986; Winstanley et al., 2005) and these transmitter systems undergo adaptations in animals that have been food deprived (Carlson et al., 1988; Haleem and Haider, 1996; Huether et al., 1997; Kohsaka et al., 1980). There is also an interest in the affects that psychostimulants have on impulsivity, particularly in light of the use of these drugs in substance abuse disorders and for treatment in attention deficits hyperactivity disorders. Food deprivation can confound results in these studies as well. For example; food deprived rats more vigorously seek out, and are more sensitive to the rewarding effects of, amphetamine and cocaine (Bell, et al., 1997; Cabeza de Vaca and Carr, 1998; Carroll et al., 1984). Thus, conclusions drawn from food reinforcement studies must take into account the adaptations known to occur with food deprivation.

We revealed that once established, probability discounting could be measured every day or every fourth day, and similar risk-taking profiles were obtained. We also demonstrated that the baseline risk-taking profile was not altered by repeated systemic injections of saline, a common vehicle used in drug studies. These findings are of technical importance showing the paradigm can be applied to long term evaluations of chronic drug treatments, and that infrequent testing is sufficient to obtain an accurate

assessment of the behavior.

Two factors influence the strength of an EBS: current intensity and frequency. We verified that the capacity to perform probability discounting is independent of stimulation current modality, such that the task is sensitive to different levels of current intensity or frequency. The value of this last milestone lies in the theoretical constructs of what neuronal elements are engaged with various stimulation parameters. With increasing current intensity, the current spread increases, and more neurons (of potentially different transmitter phenotypes) are activated (e.g., see (Maslowski and Napier, 1991; Mitrovic and Napier, 1995). In contrast, when frequency of stimuli is increased within physiological ranges and with a constant intensity, the firing rate is increased but the population of activated neurons remains consistent (Pillolla et al., 2007). We revealed that lever pressing rate was positively correlated to changes in magnitude of EBS intensity or frequency, that individualized smaller and larger rewards could be obtained and discriminated by the rats for both modalities, and that both modalities supported the probability discounting task. This agrees with findings that, when the EBS train duration is kept constant (as was done in the current study), the subjective value of reward is a product of the current intensity and frequency (Gallistel and Leon, 1991). For example, doubling the current intensity will half the frequency needed to maintain the same reward magnitude (Gallistel and Leon, 1991). Data from the current study revealed more variability among rats when collecting rate-intensity curves, as compared to rate-frequency curves. This could be explained by the fact that rats tested in the latter had completed the entire probability discounting task with current intensity and therefore, these rats were exceptionally well-trained. However, it may be related to

the modality; ongoing studies in our laboratory continue to obtain more moderate variability among rats when using the rate-frequency curves.

In conclusion, we reveal here that ICSS can serve as a positive reinforcer for probability discounting paradigm protocols. The present six phase protocol leads to stable discounting functions that persist for several weeks and allows for the assessment of chronic manipulation, such as pharmacological treatments, on risk-taking behavior.

Table 1. The six phase ICSS-mediated probability discounting paradigm; time-line and phase details.

Phase Title	Phase Training Objective	Phase acquisition criteria	Maximal # of sessions to reach criteria	Timeline for phases[a] (Days)
1. Shaping	Associate a lever press with EBS.	Steady lever pressing (~eight presses/min) on both levers	3	7-8
2. FR-1	Demonstrate steady lever pressing on both levers	Lever press ≥ five times within the first two min of the session (i.e., to initiate the session) and maintain a minimum average of eight lever presses/min in four consecutive sessions.	4	9-10
3. Rate-Current Strength[b] Function	Demonstrate a stable positive correlation between LH stimulation parameters[b] and the rate of lever pressing.	E_{max} and threshold values ±20% for three consecutive curves.	11	11-16
4. Discrete Trials	Recognize the temporal nature of 15sec trials	Complete >150 trials/session for four consecutive sessions.	6	17-19
5. Choice Test	Recognize and select from differing, lever-specific, reinforcement values (i.e., large and small reinforcer)	Select the larger of the two reinforcers in each block, for an average of ≥70% of the trials for three consecutive sessions.	14	20-26
6. Probability Discounting Task	Demonstrate a positive correlation between selection of a large and/or risky reinforcer and the probability of that reinforcer being delivered.	Data subjected to a two way rmANOVA with Day and Probability as factors. Stability defined as no effect of day, a significant effect of probability and no interaction ($p<0.1$) for three consecutive days.	3	27-37

[a] Surgery occurred on Day 0, followed by six days of recovery.

[b] Intensity (μA) or frequency (Hz).

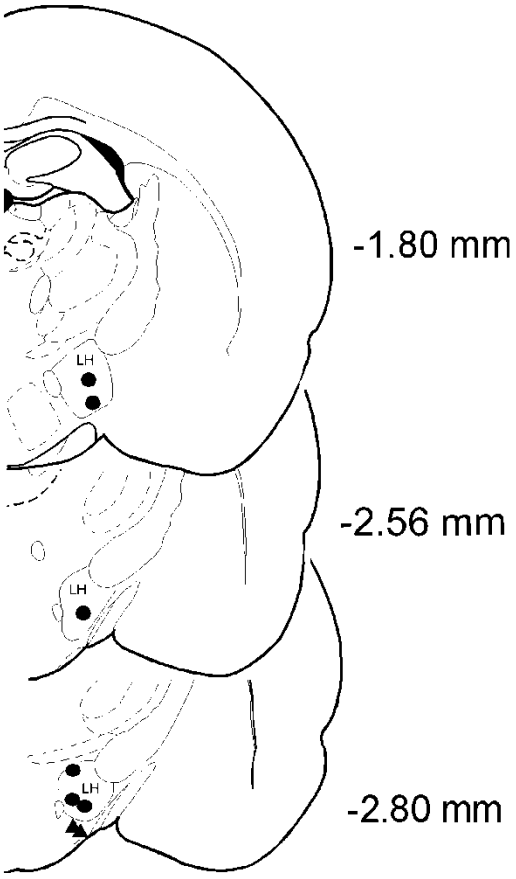


Figure 5. Illustration of electrode tips targeted at the medial forebrain bundle at the level of the lateral hypothalamus (LH). Collapsed onto three neuroanatomical plates are representations of the tip of the electrode (circles) targeted to the medial forebrain bundle (modified from Paxinos and Watson (1997)). For two of the eight rats, the electrode was implanted such that the tip was ventral to the target area (triangle) and these two rats did not perform ICSS. The numbers indicate distance from bregma.

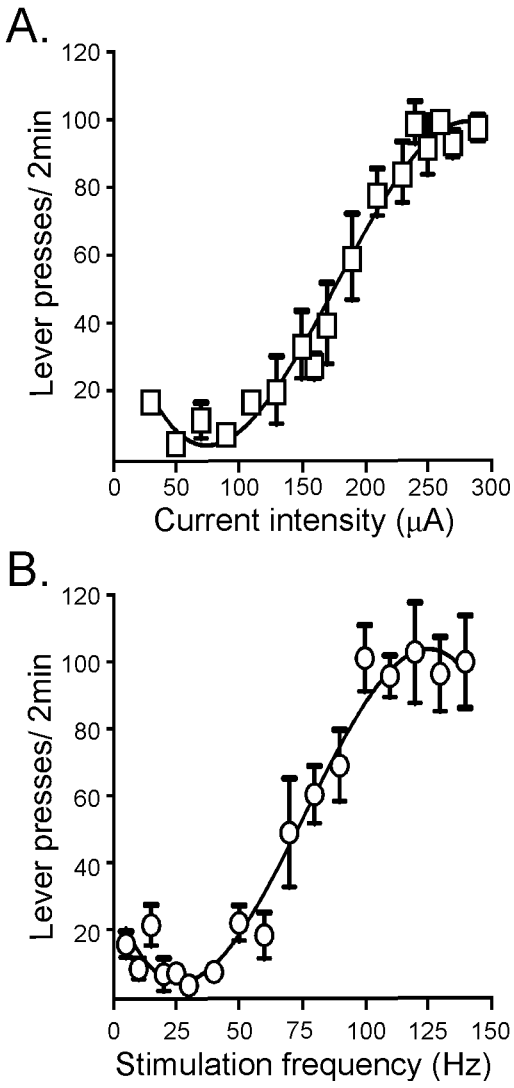


Figure 6. A representative rate-current intensity and rate-frequency function. After maintaining stable lever press responding with various current intensities (A) or stimulation frequencies (B) for three consecutive sessions, a final curve was generated for each rat tested (n=6, and 4, respectively). Shown are the mean ± SEM. The plot is drawn as a third order polynomial to help visualize E_{max}, and threshold. Determinations of $ECur_{90}$, $ECur_{60}$ and $ECur_{40}$ from this type of curve are explained in the methods section.

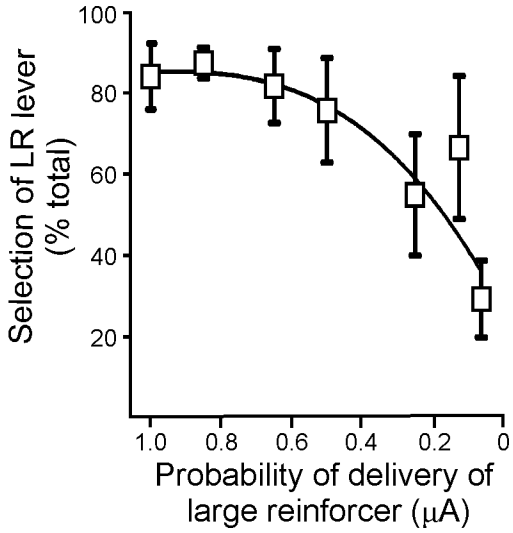


Figure 7. Acquisition of the probability discounting task in Phase 6. Shown are data taken from six rats during their first session for the discounting task. A positive correlation between the free-choice ratio (i.e., per block, number of times rats selected the large/risky (LR) lever divided by the total number of lever presses made X 100) and the probability that the large reinforcer was delivered. The plot is drawn as a third order, nonlinear regression, the statistics for which are R^2=0.37. The reinforcer options were based on current intensity. Shown are the mean ± SEM.

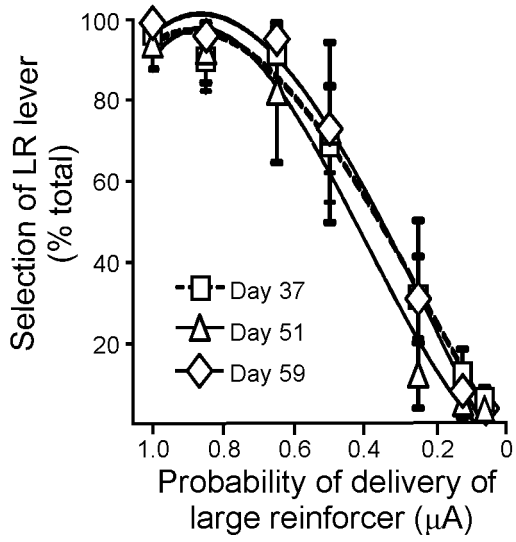


Figure 8. Persistence of stable discounting behavior. Following two weeks of periodic discounting testing during saline treatment (protocol days 38-51), rats were tested in the probability discounting task on protocol days 55 and 59. Compared to the last day of baseline discounting, day 37 (squares), stable behavior was maintained to the end of the saline treatment, day 51 (triangles), and on the last day of the study, day 59 (diamonds). Shown is the free-choice ratio (i.e., per block, number of times rats selected the large/risky (LR) lever divided by the total number of lever presses made X 100) *vs.* the probability that the large reinforcer was delivered. The reinforcer options were based on current intensity. Shown are the mean ± SEM; (n=3 rats) for each curve.

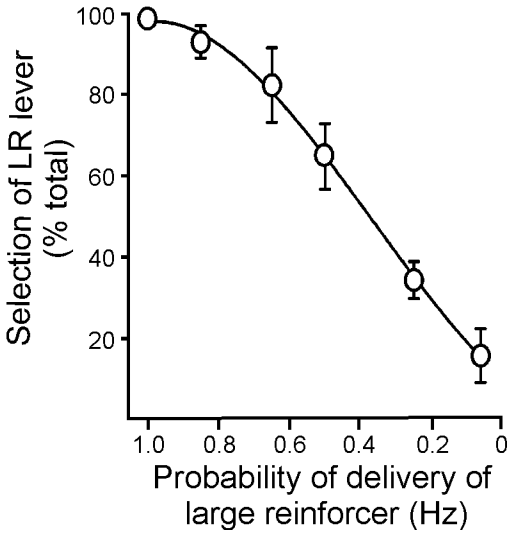


Figure 9. Probability discounting behavior using frequency (Hz) as a reward modality. Rats underwent two days of testing in the discounting task using a small and large reinforcer that differed only in current frequency of the brain stimulation. Shown is the free-choice ratio (i.e., per block, number of times rats selected the large/risky (LR) lever divided by the total number of lever presses made X 100) *vs.* the probability that the large reinforcer was delivered. Data are presented as the mean ± SEM; (n=3 rats). The profile of the discounting curve is similar to the discounting curves generated using current intensity as the reward modality (compare to Fig. 4).

CHAPTER IV

PRAMIPEXOLE ALTERED PROBABILISTIC DISCOUNTING: COMPARISONS BETWEEN A RODENT MODEL OF PARKINSON'S DISEASE AND CONTROLS

Abstract

The dopamine (DA) agonist pramipexole (PPX) can increase measures of impulsiveness, and PPX therapy for neurological diseases (Parkinson's disease (PD), restless leg syndrome) is associated with impulse control disorders (ICDs) in subpopulations of treated patients. A commonly reported ICD is pathological gambling in which risk-taking is a prominent feature. Probability discounting is a measureable aspect of risk-taking. We recently developed a probability discounting paradigm wherein intracranial self-stimulation (ICSS) serves as the positive reinforcer. Here we used this paradigm to determine effects of PPX on discounting. We included assessments of a rodent model of PD, in which 6-OHDA was injected into the dorsolateral striatum of both hemispheres and which produced persistent PD-like deficits in posture adjustment. Rats were trained to perform ICSS-mediated probability discounting, in which PD-like and control groups exhibited similar profiles. Rats were treated twice daily for two weeks with 2mg/kg (±)PPX (a racemic mixture of the drug that is equivalent to 1mg/kg of the active form), a dose that improved lesion-induced motor deficits. In both groups, PPX increased discounting; preference for the large reinforcer was enhanced 30-45% at the most uncertain probabilities. There was no difference between the two groups.

Tolerance did not develop with repeated treatments. Increased discounting subsided within two weeks of PPX cessation, and re-exposure to PPX reinstated heightened discounting. Such findings emulate the clinical scenario; therefore, ICSS for discounting assessments in rats exhibited high face validity. This model should prove useful in medication development where assessment of the propensity of a putative therapy to induce risk-taking behaviors is of interest.

Introduction

DA agonists PPX and ropinirole are FDA-approved for treatment of motor dysfunction in PD and restless leg syndrome (RLS). DA agonist therapy is associated with impulse control disorders (ICDs) in an estimated 14% of treated PD patients (Voon and Fox 2007; Weintraub et al. 2010) and 7-12% of treated patients with RLS (Pourcher et al. 2010; Driver-Dunckley et al. 2007). These drugs are being used off label for other pathologies, including fibromyalgia and bipolar disorders wherein ICDs are also observed (Holman 2009; Strejilevich et al. 2011). Independent of the pathology for which the therapy is implemented, ICD onset is reported to relate to onset of DA agonist treatment, and symptoms typically subside with dose reduction or discontinuation (Dodd et al. 2005; Driver-Dunckley et al. 2007; Mamikonyan et al. 2008; Quickfall and Suchowersky 2007). In North America, ICDs associated with DA agonists commonly include problem/pathological gambling, compulsive sexual behavior, compulsive buying, and binge-eating (Weintraub et al. 2010). These behavioral disorders are reward- or incentive-based and repetitive in nature (Evans et al. 2009), indicating that DA agonists can lead to dysregulation of general reward processes. Supporting this concept, acute

PPX can enhance reward-mediated learning (Pizzagalli et al. 2008; Santesso et al. 2009) and impulsivity in healthy human volunteers (Riba et al. 2008); but see (Hamidovic et al. 2008).

To better understand the link between DA agonists and ICDs, and to provide a means to screen new therapies without a propensity to induce aspects of impulsivity, a valid animal model is needed. Towards that end, we developed a novel probability discounting paradigm in laboratory rats (Rokosik and Napier, 2011). This task measures how changes in probabilities alter decision making. For example, subjects are given a choice between a small reward that is always delivered and a large reward that is sometimes delivered. If the probability of obtaining a large reward is high, the subject will prefer the large reward; however, lower probabilities will drive preference for the small reward that is guaranteed. If the subject discounts probability, the ability of lower probabilities to drive preference for the small certain reward will be decreased. Thus, an *increase* in discounting reflects a reduced influence or perhaps importance of the low probabilities, and the subject will exhibit preference for the large reward during both high and low probabilities for reward obtainment. This profile is reflected in a shallower slope for curves illustrating discounting behaviors (Figs 8 and 9 are examples of normal discounting curves, whereas Figs 16 and 17b show drug-induced increases in discounting). Probability discounting is a popular method to study risky decision-making, one facet of impulsivity. Problem gamblers demonstrate increased risk-taking in probability discounting paradigms (Holt et al., 2003; Madden et al., 2009; Petry, 2011).

To provide a potent, rapid, and reliable reward that allows for repeated tests of discounting, we employed intracranial self-stimulation (ICSS) as the positive reinforcer

in rats (Rokosik and Napier, 2011). The ability for repeated testing is a critical feature for assessments of chronic treatments. As yet, laboratory evaluations have not been conducted for chronic PPX administration, and this is needed to better emulate the therapy scenario used clinically. To fill this gap, the current study evaluated the effects of chronic PPX treatment on probability discounting. To emulate the pathology for which PPX is most often used clinically, we included assessments in a 6-OHDA model of PD. As DA agonists, including PPX, are front-line therapy for early stage PD (Bonuccelli et al., 2009), we sought to model the human brain at this stage, i.e., when dopaminergic lesions are largely confined to the putamen (Kish et al., 1988). The rodent dorsolateral striatum (DLS) is the homolog of the primate putamen, and lesions of DA inputs to the DLS *via* 6-OHDA injections are a common way to model early stages of PD in rats (Deumens et al., 2002; Przedborski et al., 1995). Notably, early stage PD, the DA projections that comprise the mesolimbic pathway are left relatively intact (Bernheimer et al., 1973; Kish et al., 1988). In the DLS-lesion, this pathway is left intact. It remains unclear as to why SNpc neurons are more susceptible to cell death, however there have been several potential explanations. For example, vulnerability of SNpc neurons have been related to the presence of iron (Faucheux et al., 1995; Lv et al., 2011) elevated cytosolic DA (Mosharov et al., 2009) elevated DA metabolites (Galvin, 2006) and elevated DA transporter glycosylation (Afonso-Oramas et al., 2009). On the other hand, dopaminergic neurons in the VTA are protected by the presence of calcium binding proteins such as Calbindin-D_{28k} and calretinin (German et al., 1992) and expression of transcription factors, such as Otx2 (Simeone et al., 2011) which can protect neurons from excessive amounts of calcium and DA, respectively. Moreover, SNpc neurons are

susceptible to cell death by α-synuclein (Dawson and Dawson, 2003), whereas evidence suggests that these protein aggregations do not lead to VTA neuronal degeneration (Maingay et al., 2006).

Materials and Methods

Subjects

Male Sprague-Dawley rats weighing 250-274g upon arrival (Harlan, Indianapolis, IN) were housed in pairs under environmentally controlled conditions (7:00AM/7:00PM light/dark cycle, temperature maintained at 23-25°C) with access to rat chow and water *ad libitum*. Rats were handled according to federal standards. Protocols were approved by Rush University IACUC.

Treatment drugs

Pramipexole (synthesized as the racemic mixture; Daya Drug Discoveries; Hazelwood, MO) (±PPX) was dissolved in saline and given intraperitoneally (ip) as 0.25, 0.5, 1.0, 2 or 4mg/ml/kg for assessments in stepping and 2mg/kg for the discounting task. To induce dopaminergic lesions, 6-hydroxydopamine-hydrobromide (6-OHDA; Sigma-Aldrich, St Louis, MO) was dissolved in 0.2% ascorbic acid in a sterile saline solution (pH=5.0) and infused into the striatum at a dose of 7.5µg/2µl/side (as the salt). Thirty min beforehand, rats were given 25mg/kg, ip (as the salt) of desipramine-HCl (DMI; Sigma-Aldrich, St Louis, MO) dissolved in sterile water to reduce uptake of the 6-OHDA into adrenergic neurons.

Surgical procedures for 6-OHDA injections and electrode implantation

To stereotaxically lesion the striatum and implant the stimulation electrode rats were anesthetized with sodium pentobarbital (50mg/kg/ml ip; Sigma-Aldrich, St Louis, MO), administered DMI, and the head placed in a stereotaxic frame (David Kopt, Tujunga, CA) with the nose piece set at 3.3mm below the horizontal. A 33 gauge, bilateral injector was lowered to the dorsolateral striatum (DLS; 1.0mm anterior to bregma, 3.4mm lateral from midline, 4.7mm ventral from skull). Thirty min post DMI, 6-OHDA was injected at a rate of 0.2µl/min for 10 min. Sham controls were similarly injected with the ascorbic acid vehicle. The injectors were left in place for an additional min (to allow the solution to diffuse away from the tip) and the skull holes were filled with bone wax. A bipolar stimulating electrode (MS303/3-B/SPC; Plastics One, Roanoak, VA) was lowered to the lateral hypothalamus (LH; 2.6mm posterior to bregma; 1.8mm lateral; 8.4mm ventral). Electrodes were secured to the skull with stainless steel screws and dental acrylic, and the incision was sutured. Rats were allowed at least five days recovery from surgery before operant testing was initiated.

Behavioral Testing

Motor assessment: Forelimb adjusting step test

6-OHDA-induced motor deficits were verified using the forelimb adjusting step test, (Olsson et al. 1995) conducted one day before surgery and at least once a week post-surgery. To do so, the experimenter suspended the rat's rear legs and one forelimb while the rat supported itself on its unrestrained forelimb. The rat was ‘dragged’ on the unrestrained forelimb 0.9m/5sec in abduction and adduction directions for both

forelimbs, and the number of adjusting steps was counted. Three stepping trials were taken per session, and the average score was determined.

An initial study was conducted to validate the rat model of PD employed here with regard to (i) brain DA deficits, and (ii) motor dysfunction for a time frame that would coincide with duration of the probability discounting paradigm. 6-OHDA-treated rats were sacrificed 21 days (n=6) or 60 days post-lesion (n=6); sham rats (n=5) were sacrificed 60 days post-lesion. Forelimb stepping adjustments were measured every three days. Lesion extent was verified in *ex vivo* tissue harvested 21 or 60 days after 6-OHDA infusion using tyrosine hydroxylase immunohistochemistry.

A separate group of lesioned rats (also implanted with stimulation electrodes) were used to conduct a (±)PPX dose *vs.* stepping response evaluation. These rats were tested with the stepping task one day before surgery and every week after. Approximately 40 days after the lesion, the following protocol was used: PPX was administered to sham (n=7) and 6-OHDA-treated rats (n=5) in the AM and stepping adjustments were measured immediately before, and 1 and 6hr after treatment. In the PM, a second PPX injection (of the same dose) was given and stepping was measured 17hr later. Treatments (vehicle, 0.25, 0.5, 1, 2 and 4mg/kg, ip) were administered weekly in a pseudo-randomized order.

Intracranial self-stimulation (ICSS) procedures and apparatus

ICSS experiments were conducted in operant chambers (30.5cm x 24.1cm x 21.0cm; Med-Associates, St. Albans, VT) outfitted with a chamber light, and two retractable levers each under a stimulus light and enclosed in ventilated, sound attenuated

boxes. Electrical brain stimulation (EBS) was delivered by a programmable stimulator (PHM-152/2) *via* bipolar leads connected to commutators (Plastics One, Roanoak, VA) mounted above the chamber. Typically, two ICSS test sessions were conducted per day. The following describes the testing protocols for various phases in the probability discounting paradigm:

ICSS-mediated probability discounting

A nine phase paradigm was used to determine rats' baseline discounting and effects of PPX, as previously described (Rokosik and Napier, 2011). Table 2 shows the acquisition criteria for Phases 1-6 that were required before initiating PPX treatment (Phases 7-9) in the current study. Briefly, Phase 1, Shaping. A single lever was extended and electrical brain stimulation (EBS; 200μs biphasic square wave pulses with a 100μs delay between pulses, applied at 100Hz for 500ms) was delivered. Only the initial current intensity (100μA) was adjusted for each rats based on their performance to approach and ultimately press the lever. The final intensity level was used for the remaining Phases. Phase 2, Fixed ratio-1 (FR-1) reinforcement. To establish stable ICSS lever pressing, rats underwent a continuous FR-1 reinforcement schedule wherein one lever was extended for a 30min session. Phase 3, Rate-Frequency Function. Rats were pseudo-randomly presented with one of 16 different current frequencies tested in 10Hz increments, ranging from 10-160Hz. Train duration and current intensity were held constant. For each frequency, rats had access to the lever for 2min and the number of lever presses were recorded. Following each 2min period, the lever retracted for 10sec. In each session, a lever pressing rate *vs.* ICSS current frequency (termed the *rate-*

frequency function) was collected and the maximal (E_{max}) and minimal (threshold) number of lever presses were determined using a non-linear regression (GraphPad Prism, La Jolla, CA). When a rat met phase acquisition criteria (see Table 3), averages of three curves were used to determine ICSS frequencies that produced 90%, 60% and 40% of E_{max} (termed *effective current* (ECur); $ECur_{90}$, $ECur_{60}$ and $ECur_{40}$, respectively; see Fig. 10). Phase 4, Discrete Trials. Rats were trained to recognize the temporal nature of trials using each rat's own $ECur_{60}$ as the reinforcer. Each session was comprised of 200 trials. Trials occurred in 15sec intervals. Each session began with both levers retracted and the chamber light off; 2sec later, the chamber light was illuminated, followed 3sec later by the extension of one lever. The rat had 10sec to press the lever, if the response was not executed, the trial was aborted (termed an *omitted trial*), the lever retracted and the chamber light turned off. If a lever press was made, an EBS was delivered and the stimulus light over the lever was turned on. After 0.5sec, all lights were turned off and the lever retracted. The two levers were alternately extended among trials. Phase 5, Choice Test. The purpose of this Phase was to determine for each rat, a small and large reinforcer that could be used in the probability discounting phase. Using the FR-1 discrete trials described in Phase 4, rats were trained to select from different, lever-specific, reinforcement values. Each session consisted of three blocks. Each block consisted of 20 forced-choice trials followed by 20 free-choice trials. In forced-choice trials, one lever was extended at a time allowing the rat to learn the reinforcement value associated with that lever. In free-choice trials, both levers were extended, and the rat had to choose between the lever-specific reinforcement values. Initially, small and large reinforcers corresponded to the rat's $ECur_{90}$ and $ECur_{40}$ (obtained in Phase 3). To

complete this phase, rats had to demonstrate a "*free-choice ratio*" (the number of selections for the large reinforcer divided by the total number of lever responses made x100) of at least an average of 70% across the three blocks. Phase 6, Probability Discounting Task. Each session consisted of nine blocks as used in Phase 5, but here, one lever was designated "small/certain lever" (SC) and the other was "large/risky" lever (LR). A press on the SC lever always delivered the small reinforcer (i.e., approximately $ECur_{40}$); a press on the LR lever delivered the large reinforcer (approximately $ECur_{90}$) with varying probabilities. The following three series of probability presentations were cycled during this, and subsequent phases: (i) 0.5, 0.3, 0.85, 0.6, 0.05, 0.7, 1.0, 0.4 and 0.15; (ii) 0.15, 0.6, 0.4, 0.05, 0.7, 0.3, 0.85, 1.0 and 0.5; and (iii) 0.7, 0.4, 1.0, 0.15, 0.5, 0.85, 0.05, 0.3 and 0.6. For each series, the LR lever was designated either to the left or right lever; therefore, each rat experienced six different probability formats. Data from free-choice trials of each probability (i.e., block) were analyzed to determine a baseline free-choice ratio *vs.* probability function. If in a block, there were 50% or more omissions from the free-choice trials (i.e., more than 10 of 20 trials tested), data from that block were excluded from subsequent analysis. This criterion was held for Phases 6-9 (each of which employed the probability discounting task), and overall, less than 2% of the blocks were excluded. Phase 7, (±)PPX treatment. One day following the last baseline test, PPX treatment was initiated. The regimen was 2mg/kg (±)PPX, ip, twice a day (in the AM and PM) for 13 days (termed, *chronic treatment*). PPX-induced changes in discounting were assessed 30min and 6hr following the AM injection on the first and every third day of the chronic treatment. Phase 8, withdrawal. In a subset of rats, PPX was withdrawn for 15 to 69 days after cessation of treatment. Phase 9, re-instatement.

PPX treatment was reinitiated twice a day for seven days. Probability discounting was assessed every third morning throughout Phases 8 and 9.

Histology and tyrosine hydroxylase immunohistochemistry (TH-IHC)

Rats were deeply anaesthetized with chloral hydrate (400mg/kg; Sigma, St. Louis, MO). A 5V DC current was applied to the stimulating electrode for 30sec to deposit iron and/or produce a very discrete lesion at the electrode tip. The iron deposits were visualized by a blue coloration produced *via* trichloroacetic acid (0.5%) and potassium ferricyanide (3%) added to a 4% paraformaldehyde solution used for transcardial perfusion after perfusing with ice cold 0.9% NaCl. Brains were removed, post-fixed in 4% paraformaldehyde and stored in a 30% sucrose solution. Brains were sliced in 40μm coronal sections. Striatal sections were immunoreacted with a primary monoclonal mouse anti-TH antibody (ImmunoStar, 22941) diluted 1:10,000 and a biotinylated horse anti-mouse IgG (Vector Laboratories, BA2001) diluted 1:100. The signal was amplified by avidin and biotinylated horseradish peroxidase using the Elite ABC Vectastain Kit (Vector Labs, PK6100). Immunostaining was visualized with 3,3-Diaminobenzidine tetrachloride dehydrate (Sigma, D5637) solution activated with 0.3% H_2O_2.

Data analysis

To compare PD-like and control rats, data from Phases 1-6 were analyzed using a Student's *t*-test. A linear correlation was conducted between (i) lever pressing rate and EBS frequency (Hz; Phase 3) to verify that changes in EBS frequency altered ICSS, and (ii) free-choice ratio and probability magnitude (Phase 6) to determine if the two groups

acquired the discounting task. To determine treatment-induced changes in free-choice ratio collected in Phases 6-9, a two way repeated measures (rm) ANOVA was conducted. For Phases 6-7, day and probability were factors. For Phases 8-9, phase and probability were factors. A *post hoc* Newman-Keuls provided individual comparisons. Forelimb stepping was similarly analyzed with time and dose as factors. If a data point exceeded two standard deviations from the group mean, it was considered an outlier and it was excluded from analysis. Significance was $p<0.05$ for group/treatment comparisons; data are reported as group means ± SEM.

Results

Intra-Dorsolateral Striatal Injections of 6-OHDA Produced Persistent Motor Deficits that were Reversed by Pramipexole

We conducted an initial study to validate that the DLS infusions of 6-OHDA resulted in a lesion that was sufficiently robust and persistent to produce stable and enduring reduction of TH in the DLS and in deficits in forelimb stepping, similar to a previous report (Chang et al., 1999). The DLS of 6-OHDA-treated rats showed profound reductions in TH staining that persisted for 60 days (Fig. 11). For the six rats killed at 21d post lesion, the tissue sections which showed the largest lesion extent were between +1.2mm to +0.7mm anterior to bregma, and the lesion could be detected from +2.2mm to -0.26mm. While all rats had similar pre-surgery baseline stepping, those treated with 6-OHDA displayed stepping deficits in both left and right forelimbs when tested in both the adduction and abduction direction. These deficits, which were similar for 21 and 60d

post lesion, and were about 40-50% of that obtained from sham rats (see Table 3). These data were analyzed using a planned contrast two way rmANOVA. For all four parameter tested, there was a significant ($p<0.05$) effect of treatment group and post-surgery time, and group by time interactions (Left abduction: group $F_{(2,48)} = 13.63$, time $F_{(1,48)} = 193.95$, interaction $F_{(2,48)} = 20.84$. Right abduction: group $F_{(2,48)} = 14.21$, time $F_{(1,48)} = 142.02$, interaction $F_{(2,48)} = 15.78$. Left adduction: group $F_{(2,48)} = 12.30$, time $F_{(1,48)} = 146.04$, interaction $F_{(2,48)} = 40.01$. Right adduction: group $F_{(2,48)} = 3.30$, time $F_{(1,48)} = 54.83$, interaction $F_{(2,48)} = 18.37$.) This study verified that the 6-OHDA treatment protocol profoundly reduced dopaminergic innervation of the DLS and that this lesion was sufficiently robust and enduring to produce stable deficits in motor function that persist for at least 60 days. Thus, this 6-OHDA treatment protocol was employed for the subsequent ICSS studies, and stepping adjustments of the left forepaw in the abduction direction were used as the representative motor index of the DLS lesion.

To verify that the deficits remained throughout the 85 days needed to complete the study, a separate group of rats that completed the ICSS-mediated discounting paradigm (n=21) were also assessed for forelimb stepping each week post-surgery. We determined that stepping remained at approximately 17 steps/session for control rats and at 4-5 for PD-like rats. Similar to the rats tested 60d post lesion (discussed above), these motor deficits persisted throughout the study (i.e., for 85 days, data not shown).

To evaluate the ability of PPX to reverse 6-OHDA-induced motor deficits, rats that failed to meet acquisition phase criteria in the discounting paradigm (n=5/16 PD-like; 7/17 shams; refer to Table 2 for criteria) were used. For these rats, the pre-surgery baseline average of adjusting steps/session were 14-15 and this level was not altered in

control rats by either vehicle treatment or any dose of (±)PPX tested (data not shown). In contrast, PD-like rats showed a significant effect of (±)PPX dose ($F_{(5,20)}$ =34.17, $p<0.01$) and post-treatment time ($F_{(3,60)}$ =316, $p<0.01$) and an interaction ($F_{(15,60)}$ = 46.88, $p<0.01$). As shown in Fig. 12, at doses ranging from 0.5-4.0mg/kg ip, (±)PPX improved stepping deficits in PD-like rats at 1hr post treatment; 1.0-4.0mg/kg maintained stepping improvements for at least 6hr post treatment. Adjusting steps returned to pre-(±)PPX deficit levels 17hr after injection for all doses tested. The stepping deficit was not altered by vehicle or 0.25mg/kg (±)PPX. The 2mg/kg (±)PPX dose produced robust motor improvements that persisted for 6hr and yet was below maximal improvement seen. Furthermore, this dose was not sufficient to influence behavior at 17hr post injection (Fig. 12). Therefore, the treatment given in the late afternoon was mostly cleared from the animals before the morning injection, and PPX likely did not accumulate during the repeated injections. These outcomes guided the dosing protocol selected for the probability discounting paradigm, i.e., 2mg/kg (±)PPX (i.e., 1mg/kg of the active form), administered twice a day. This decision was also guided by reports that (i) 1mg/kg of (-) PPX alters reward-mediated behavior, i.e., enhances the reinforcing effects of cocaine (Caine et al., 1997) and (ii) twice-daily injections of 1mg/kg of (-)PPX in rats increases expression of forebrain D3 receptors (Maj et al., 2000), which are involved in ICDs and addictions (Heidbreder and Newman, 2010).

PD-like and Control Rats Performed Similarly in the Probability Discounting Paradigm

Post mortem histological evaluations verified that rats completing the ICSS-mediated paradigm had electrode tip placements located in the lateral hypothalamus (Fig. 13). To determine if PD-like rats differed from controls in any aspect of paradigm acquisition, performance in Phases 1-6 was monitored and compared for the two groups (refer to Table 3). All rats quickly acquired stable ICSS lever pressing, and both groups lever pressed on an FR-1 at similar rates. Likewise, the $ECur_{90}$, $ECur_{60}$, and $ECur_{40}$ obtained from each rat's ICSS rate *vs.* current frequency curve did not differ between groups. The averaged rate-frequency functions for PD-like and control rats are graphically indicated in Fig. 14. Both groups exhibited significant linear regressions; PD-like, $r^2=0.94$, $p<0.01$; and control rats $r^2=0.91$, $p<0.01$. The two groups learned and met phase criteria for the discrete trials and the choice tests in a similar time frame (Table 2). All rats that entered Phase 6 were able to learn the discounting task. Fig. 15 illustrates that both groups acquired the probability discounting task in the first session of Phase 6, as demonstrated by a reduction in selection the LR lever as the probability for delivery of the large reinforcer decreased (PD-like rats: $r^2=0.73$, $p<0.01$; control rats: $r^2=0.85$, $p<0.01$). While the range for individual rats to obtain stable baseline discounting was three to six days, as groups, both the PD-like and control met stability criteria in the first three test days. For these three days of discounting, control rats showed an effect of probability ($F_{(8,216)} = 47.89$, $p<0.01$) but no day effect ($F_{(2,27)} = 0.32$,$p=0.73$), nor interaction ($F_{(16,216)} = 1.17$, $p=0.29$). There were 7 data points removed due to meeting

statistical outlier criteria. Likewise, for PD-like rats, there was an effect of probability ($F_{(8,240)} = 62.6$, $p<0.01$), without an effect of day ($F_{(2,30)} = 0.23$, $p=0.80$) or interaction ($F_{(16,240)} = 1.3$, $p=0.20$). There were 8 data points removed due to meeting statistical outlier criteria. Thus, for both groups there was a direct relationship between reward probability and free-choice ratio which did not differ for the first three baseline test days. For this Phase, 9 of 1,134 total blocks had response omissions of 50% or more, and were omitted from the free choice ratio analyses.

Pramipexole Increased Discounting in the Probability Discounting Task

To determine if PPX altered probability discounting, rats were treated with 2mg/kg (±)PPX twice a day (approximately 8AM and 5PM) for 13 days during Phase 7. Discounting was measured 30min and 6hr after the AM injection approximately every three days. These data were compared to pretreatment baseline sessions which were similarly conducted twice a day. Thus, to control for the possible effects of time of day for testing on outcomes, AM baseline sessions were compared to the tests taken 30min after PPX (also an AM test), and PM baseline sessions were compare to the tests taken 6hr after the AM PPX injection (refer to Fig. 16). For PD-like rats, comparisons of free-choice ratio for AM baseline to 30min after the 1st and 25th (±)PPX injection revealed enhanced discounting (Fig. 16A). There was a significant effect of test (i.e., baseline, 1st and 25th injection of ±PPX; $F_{(2,29)} =14.45$, $p<0.01$) and probability ($F_{(8,232)} =31.07$, $p<0.01$) and an interaction ($F_{(16,232)} =5.85$, $p<0.01$). Likewise, comparison of PM baseline to 6hr following the 1st and 25th PPX injection revealed that PPX-induced heighted discounting was sustained (Fig. 16C), with a significant effect of test ($F_{(2,30)} =28.78$,

$p<0.01$), probability ($F_{(8,240)} =65.42$, $p<0.01$) and the interaction ($F_{(16,240)} =6.49$, $p<0.01$). A *post hoc* Newman-Keuls comparison revealed that discounting was most pronounced following the 25th (±)PPX treatment for both 30min and 6hr post-injection (Fig. 16). Unexpectedly, some control rats exhibited a large number of trial omissions 30min following the first treatment of PPX. Observation of these rats in the operant boxes revealed they were engaged in continuous stereotypic sniffing and licking of the floor metal bars, with some head bobbing. The behaviors abated 6hr after the PPX injection. The rats became tolerant to the motor effects, for on the 4th day of treatment (and the second discounting test) they were fully engaged in the lever pressing task and discounting performance could be accurately evaluated. However, the acute motor confound precluded discounting assessments for the first, 30min post-PPX treatment in control rats. After the 7th injection (i.e., the 4th day of PPX treatment), control rats clearly demonstrated increased discounting as the selection for the risky lever at the 0.05, 0.15 and 0.30 probabilities were greater than baseline by 31%, 25% and 27%, respectively. Fig. 16B illustrates the enhancement in discounting observed 30min after the 25th injection for control rats. There was a significant effect of test ($F_{(1,18)} =4.97$, $p=0.04$) and probability ($F_{(8,144)} =20.93$, $p<0.01$) and an interaction ($F_{(8,144)} =3.22$, $p<0.01$). Comparison of PM baseline testing to the 1st and 25th injection for the 6hr period also showed a significant increase in risky behavior (Fig. 16D), with a significant effect of test ($F_{(2,26)} =22.34$, $p<0.01$), probability ($F_{(8,208)} =42.21$, $p<0.01$) and an interaction ($F_{(16,208)} =2.18$, $p<0.01$). As illustrated in Fig. 16D, a *post hoc* Newman-Keuls comparison revealed that discounting was most pronounced following the 25th PPX treatment. For this Phase, 50 out of 2,457 total blocks had response omissions of 50% or more, and were

omitted from the free choice ratio analyses.

To help interpret PPX-induced changes in probability discounting, we evaluated the effects of the agonist on various behaviors that are critical for the discounting task. First, demonstrating that rats maintained their ability to discriminate among the reinforcement values (i.e., no reward *vs.* small reward *vs.* large reward), we determined at various times during the PPX treatment that responding in the Choice Test (i.e., the Phase 5 protocol) was preserved (i.e., selection for the larger reinforcer was approximately 70% or higher) for both PD-like (n=9) and control (n=8) rats. Second, we determined the ability of PPX to alter the reward values. Following the 13 days of (±)PPX in Phase 7, a subset of rats (controls, n=5 and PD-like, n=3) continued to received 2mg/kg (±)PPX twice a day for three additional days and the lever pressing rate *vs.* ICSS current frequency (i.e., the Phase 3 protocol) was assessed. The $ECur_{90}$ was similar between baseline (as determined in Phase 3) and chronic PPX for both groups (controls paired *t*-$test_{(4)}$=0.89, p=0.43; PD-like *t*-$test_{(2)}$=2.5, p=0.13). However, PPX increased the rate of lever pressing at the lowest ICSS frequencies with a decrease in apparent threshold for both groups (data not shown) and for the PD-like group there was an associated reduction in $ECur_{40}$ (paired *t*-$test_{(2)}$=7.35, p=0.02). This shift went from 100Hz at baseline to 52Hz after the 32nd PPX treatment. Such a change was not seen in control rats (paired *t*-$test_{(4)}$=1.2, p=0.3) . As a collective, these evaluations indicated that even though the value of the small reward may have been enhanced by PPX, the rats continued to recognize the $ECur_{40}$ as less than the $ECur_{90}$ so as to correctly execute the Choice Test and linked Discounting Test throughout the chronic PPX treatment protocol.

Discontinuation of Pramipexole Decreased Probability Discounting

Following discontinuation of PPX treatment, rats were continually assessed for discounting in Phase 8. No overt behavioral indices of withdrawal were observed (e.g., body weight, grooming), and hereafter the term 'withdrawal' is used to indicate the absence of drug treatment, not a behavioral index. Three days following the last injection, both control and PD-like rats maintained an increase in preference for the LR lever; however, reductions in this LR lever preference were evident 15 days after treatment cessation. Within this time period, some rats began to show a decrease in general performance and omissions during the discounting task increased (i.e, more than 10 omitted trials out the 20 total); therefore, these rats were removed from the study. Of the rats that maintained performance, eight were PD-like and three were controls. For the PD-like rats, after 15 days of PPX withdrawal, selection for the LR lever decreased as compared to 30min after the 25th PPX injection (Fig. 16A). There was a significant effect of Phase (i.e., withdrawal *vs.* 25th PPX injection; $F_{(1,14)}$ =7.29, p=0.02), probability ($F_{(8,112)}$ =16.96, p<0.01) and an interaction ($F_{(8,112)}$ =2.24, p=0.03). Indeed, discounting during this withdrawal time was nearly indistinguishable from baseline behavior; at the three lowest probabilities (i.e., 0.05, 0.15 and 0.3), rats respectively selected the LR lever 52%, 55% and 59% of the time during baseline and 42%, 55% and 65% during withdrawal from chronic PPX. As illustrated in the inset of Fig. 17A, the three control rats demonstrated similar reduction in discounting as observed in the PD-like rats. That is, after 15 days of withdrawal, control rats selected the LR lever 44%, 29% and 43% of the time at the three lowest probabilities (i.e., 0.05, 0.15 and 0.3, respectively), which was similar to baseline values of 39%, 50% and 56%, respectively. During this PPX

withdrawal period, 4 out of 198 total blocks had response omissions of 50% or more, and were omitted from the free choice ratio analyses.

Re-initiation of Pramipexole Reinstated Increased Discounting

A subset of drug-withdrawn rats (n=6; all PD-like) maintained successful performance of the discounting task and thus were continually tested up to 69 days post treatment. Throughout this time period, discounting remained near baseline levels (Fig. 17B). Subsequently, the twice daily 2mg/kg (±)PPX treatment was reinitiated. The increase in discounting was reinstated by the 7th day of treatment (i.e., after the 13th injection) (Fig. 17B), with a significant effect of Phase (i.e., withdrawal *vs.* reinstatement; $F_{(1,10)}$ =6.38, $p<0.03$), probability ($F_{(8,80)}$ =10.06, $p<0.01$) and an interaction ($F_{(8,80)}$ =5.86, $p<0.01$). The increase in discounting seen with reinstatement of PPX was very similar to that obtained during the initial PPX treatment. Indeed, at the three lowest probabilities (i.e., 0.05, 0.15 and 0.3) during the initial PPX treatment, 30min after the 25th injection, rats respectively selected the LR lever 77%, 72% and 90% of the time which is comparative to 79%, 90% and 84% (respectively) taken 30min after the 13th reinstatement injection. During the reinstatement assessments, 1 out of 54 total blocks had response omissions of 50% or more, and these were omitted from the free choice ratio analyses.

Discussion

Probability discounting is a popular method to study risky decision making, and problem gamblers demonstrate increased discounting in these paradigms (Holt et al.,

2003; Madden et al., 2009; Petry, 2011). The current study utilized our new rat model of probability discounting that employs ICSS as the positive reinforcer (Rokosik and Napier, 2011), to reveal that PPX increased discounting. We also revealed that tolerance did not develop with repeated treatments, and responding was comparable between PD-like and control rats. Additionally, we verified that increases in discounting returned to baseline levels within two weeks of PPX treatment cessation, and re-exposure to PPX reinstated heighten discounting. These outcomes are in line with clinical reports wherein ICD onset is related to onset of DA agonist treatment, and symptoms typically subside with dose reduction or discontinuation (Dodd et al., 2005; Driver-Dunckley et al., 2007; Mamikonyan et al., 2008; Quickfall and Suchowersky, 2007). Thus, using ICSS for risk assessments in rats exhibits high face validity to the human experience with PPX.

ICSS provides an immediate and robust reward that does not suffer from satiety/tolerance, nor cause any withdrawal-like symptoms. Using ICSS, as opposed to food reinforcement, proved to be exceptionally advantageous for evaluating the effects of chronic PPX treatment on probability discounting. First, the ICSS-mediated discounting task was acquired by rats in the first test session, and stable baseline discounting was achieved in three days of testing. This contrasts food reinforcement discounting where typically 10 test sessions are needed for acquisition and 25-35 days are required to reach stable discounting behavior (St Onge et al., 2010; Ghods-Sharifi et al., 2009). Second, ICSS allows for testing several probabilities in a randomized order, a feature that is not successfully implemented with food-reinforced discounting (St Onge et al., 2010). Randomization encourages rats to continue selecting the LR lever even at very low probabilities (in contrast to what is obtained with protocols using predictable, descending

probabilities) (Rokosik and Napier, 2011). Thus, we were able to detect both increases and decreases in selection of the LR lever at the lowest probabilites, where the most robust discounting often occurs. Finally, in food reinforcement studies, animals typically are food-deprived to motivate them to perform the operant tasks. Food-restriction alters the behavioral effects of PPX (Collins et al., 2008) which could confound outcomes of discounting tests with the agonist. To summarize, ICSS afforded a means to unambiguously assess discounting during chronic drug administration, following subsequent, cessation of treatment, and drug reinstatement, all in the same test subjects.

The current study demonstrated the ability of a rodent model of PD to perform a probability discounting task. Although PD-like rats were robustly and persistently impaired in the forelimb adjusting step test, they readily performed the lever-pressing tasks and they did not show any behavioral deficiencies in the acquisition or execution of the discounting paradigm. Moreover, the PD-like rats displayed similar profiles as controls with regard to the reinforcing properties of ICSS currents (as assessed in the lever pressing rate *vs.* current frequency profiles) and basal discounting. These observations indicate that DA deafferentation of the DLS does not alter the capacity, or motivation, to perform ICSS-mediated probability discounting.

Acute PPX treatment in healthy humans can increase measures of impulsiveness (Riba et al., 2008) as well as disrupt reward-related learning (Pizzagalli et al., 2008; Santesso et al,. 2009), (but see also (Hamidovic et al., 2008)). As a therapeutic agent, PPX can promote problem gambling independent of the pathology for which the drug is prescribed (e.g., PD (Seedat et al., 2000; Weintraub et al., 2010), RLS (Quickfall and Suchowersky, 2007; Tippmann-Peikert et al., 2007), fibromyalgia (Holman, 2009), and

bipolar depression (Strejilevich et al., 2011)). It is unclear if these pathological conditions render individuals more susceptible to the impulsivity-related effects of PPX. Given that PPX is highly prescribed during the early stages of PD and reports suggest these patients have a relatively high incidence of PPX-induced ICDs (Weintraub et al., 2010), we included a model of PD in the current study. However, we demonstrated here that a brain state that models aspects of early stages of PD did not render rats more sensitive to the PPX-induced effects. It should be noted that this lack of differentiation between PD-like rats and controls may reflect the relatively high dose of PPX studied; lower doses of the agonist may be able to discriminate the two groups. Our findings that PPX increased discounting in control rats are in line with food-reinforcement studies using food restricted intact laboratory rats, wherein PPX increases preference for a gambling-like schedule of reinforcement (i.e., variable ratio) (Johnson et al., 2011). These converging preclinical findings support a link between PPX treatment and alterations in decision-making in regard to discounting.

In humans tested in probabilistic choice tests, PPX can disrupt learning from negative outcomes (i.e., when a reward is expected but not delivered) (Cools et al., 2006; Bodi et al., 2009). In probability discounting, when the probability of delivery of the large reinforcer is very low (e.g., 0.05, 0.15 and 0.3), the likelihood of not receiving a reward is at the highest. Negative outcomes during these low probabilities likely lessen the appeal of lever pressing for the large reinforcer and shift preference to the SC lever. This profile was seen in the current study for tests during baseline and withdrawal. In contrast, PPX enhanced responding on the risky lever during low probability. This outcome is consistent with the agonist reducing the negative consequences of a non-

rewarded response. A similar outcome might be predicted if PPX reduced the value of ICSS reward; however, the $ECur_{90}$ (current level used for the rats' large reward) was not altered by chronic PPX and the $ECur_{40}$ (the small reward) was slightly elevated. While we have recently determined with a condition place preference paradigm that PPX can support reward-mediated associated learning (Riddle et al., 2012), outcomes from the current operant task suggest that PPX may increase discounting by reducing the perceived negativity of *un*rewarded operant responses rather than enhancing the value of the reward associated with the risky lever. This interpretation is supported by clinical studies with functional magnetic resonance imaging (fMRI) that investigated the influence of PPX on reward prediction errors during a gambling task. A positive reward prediction error occurs if an unpredicted reward is encountered and negative reward prediction error occurs if a predicted reward is omitted. In one study, PD patients treated with PPX showed a correlation between increases in risk-taking and impairments in the deactivation of the fMRI signal in the orbitofrontal cortex during trials with a negative prediction error (van Eimeren et al., 2009). This suggests that the subjects were impaired from learning in trials in which losing occurred. In another study, RLS patients treated chronically with DA agonists, including PPX, demonstrated increases in fMRI signaling in the ventral striatum during trials in which expected rewards were omitted (Abler et al., 2009). It is noteworthy that the PPX-induced effects were observed in all RLS patients tested, similar to the ability of PPX to enhance discounting in all rats tested in the current study. Nevertheless, none of the RLS patients developed an ICD (Abler et al., 2009). This outcome underscores the fact that enhancement in discounting or risky behaviors is

not equivalent to developing an ICD *per se* but likely represents a particular aspect of these complicated disorders.

Which receptors mediate the behavioral effects of PPX is unclear. PPX is a direct acting DA agonist with a preference for the D3R subtype of DA receptors. For example, *in vivo* rat studies using presumed D2R- and D3R- selective behavioral assays (i.e., hypothermia and yawning, respectively), PPX is ~30 fold selective for D3R over D2R (Collins et al., 2007), 1.0mg/kg (-)PPX is sufficient to activate both D2R and D3R (Collins et al., 2007; Collins et al., 2005; Collins et al., 2009). Thus, it is likely that both subtypes were engaged by 2mg/kg dose of (±)PPX used in the current study. Indeed, both D2 and D3R have been implicated in reward-mediated behaviors (Heidbreder et al., 2005; Self, 1998) and impulsivity (St Onge and Floresco, 2009; van Gaalen et al., 2009; Buckholtz et al., 2010). Additional probability discounting studies including those with lower doses of PPX as well as receptor-subtype selective antagonists would aid in elucidating the particular receptor(s) involved in PPX-induced enhancement in discounting.

PPX shifted discounting in PD-like rats with a single injection; however, repeated treatments were required to reach maximal discounting. These findings indicate that acute occupation of relevant DA receptors is sufficient to enhance discounting; however, the adaptations in this system that were imposed with chronic administration may promote the effect. Chronic PPX treatments can lead to desensitization of DA neuronal D2/D3 autoreceptors (Chernoloz et al., 2009) and an increase in expression of D3R in dopaminoceptive regions (Maj et al., 2000). Whatever the mechanism, the neuroadaptations were reversible in the current study, for when PPX treatment was

discontinued for two weeks, discounting decreased near baseline levels. These findings concur with clinical reports showing that DA agonist-induced ICDs in humans can be eliminated with drug discontinuation (Macphee et al., 2009; Mamikonyan et al., 2008; Quickfall and Suchowersky, 2007; Dodd et al., 2005; Driver-Dunckley et al., 2007).

In summary, converging evidence suggests that PPX can influence the processing of rewards and drive decision making towards higher discounting and more risky choices. The animal model of PPX-induced discounting presented here provides a valuable new means to elucidate the pharmacological and neurobiological underpinnings of this aspect of impulsivity. This model should prove useful in the development of novel therapeutics devoid of enhancing discounting as well as a means to screen current and future compounds for their potential to promote risky behaviors.

Table 2. Phase 1-6 descriptions of acquisition phase criteria and comparisons of behavioral outcomes between PD-like and control rats.

Phase Title	Phase acquisition criteria	Behavioral Measurements			*p*
			Sham Rats	**PD-like Rats**	
1. Shaping	Associate a lever press with EBS.	No. of sessions to acquire task:	1-2	1-2	0.24
2. FR-1	Initiate lever pressing and maintain a minimum average of eight lever press/min in four consecutive sessions.	Lever presses/min Left lever: Right lever: Current amplitude:	 24±4 25±4 100-280	 25±3 23±2 160-260	 0.82 0.61 0.19
3. Rate-Frequency Function	Demonstrate stable behavior, i.e., E_{max} and threshold values ±20% of the mean for three consecutive curves.	ECur (Hz) $ECur_{90}$: $ECur_{60}$: $ECur_{40}$:	 117±14 90±11 81±10	 135±8 101±6 89±5	 0.29 0.35 0.41
4. Discrete Trials	Demonstrate completion of more than 150/200 trials per session for two consecutive sessions.	No. of sessions to acquire task:	2-6 4±1	2-7 3±1	0.65
5. Choice Test	Select the larger of the two reinforcers in each block, for an average of at least 70% of the trials for three consecutive sessions.	Preference for large reinforcer (%) Block 1: Block 2: Block 3: No. of sessions to acquire task:	 89.2±0.02 92.3±0.02 82.9±0.02 6-15 10±1	 92.2±0.02 91±0.02 86.8±0.01 5-10 8±1	 0.35 0.63 0.18 0.06
6. Probability Discounting Task	Demonstrate stable discounting. Data from daily curves were subjected to a two way rmANOVA with Day and Probability as factors. Stability defined as, no effect of Day, significant effect of probability, no interaction	No. of sessions to stability:	3-5 3±0.2	3-6 4±0.3	0.39

	($p<0.1$) for three consecutive days.				

Behavioral measurements in each phase were compared between groups using a Student's *t*-test, $p<0.05$. Data are shown as a range or as means ± SEM. For Phase 2, lever pressing rates for the last two sessions were averaged for each rat. For the three blocks shown in Phase 5, the reinforcer associated with the left lever changed from no EBS (i.e., no reinforcer), to $ECur_{90}$, to $ECur_{40}$, the right lever changed from $ECur_{40,}$ to no EBS, to $ECur_{90}$, respectively.

Table 3. Abduction and adduction forepaw adjusting steps in sham controls (n=5) and 6-OHDA-treated PD-like rats sacrificed either 21 (n=6) or 60 (n=6) days post-surgery. Comparisons were made between one day before surgery (pre-surgery) and end of study for each behavior.

ABDUCTION	Sham (60d post-surgery)	6-OHDA-treated (21d post-surgery)	6-OHDA-treated (60d post-surgery)
Left forepaw:			
pre-surgery	15±1	15±1 ^{NS}	16±1 ^{NS}
end of study	12±1	6±2*	6±1*
Right forepaw:			
pre-surgery	14±1	15±1 ^{NS}	15±1 ^{NS}
end of study	12±1	5±1*	7±2*
ADDUCTION			
Left forepaw:			
pre-surgery	11±1	12±1 ^{NS}	10±1 ^{NS}
end of study	10±1	3±1*	5±2*
Right forepaw:			
pre-surgery	11±1	13±1 ^{NS}	12±1 ^{NS}
end of study	11±1	6±1*	7±2*

Stepping data were collected at a rate of 0.9m/5sec. Data are presented as mean ±SEM. Data were analyzed with a two way rmANOVA. Superscripts indicate planned contrasts analyzed with Newman Keuls, no significant (NS) difference ($p>0.05$) *vs.* sham pre-surgery and *$p<0.01$ *vs.* sham end of study.

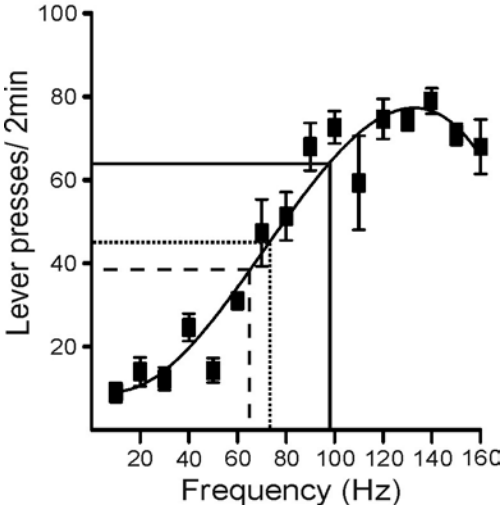


Figure 10. ICSS rate-frequency function. In Phase 3, the relationship between ICSS lever pressing rate and stimulation frequency (an index of signal strength) was obtained for each rat. Illustrated is the final curve (i.e., met stability criteria as described in Table 1) for an individual PD-like rat. From this curve, the $ECur_{90}$ (solid line), $ECur_{60}$ (dotted line), and $ECur_{40}$ (dashed line) were determined using a non-linear regression (GraphPad Prism, La Jolla, CA).

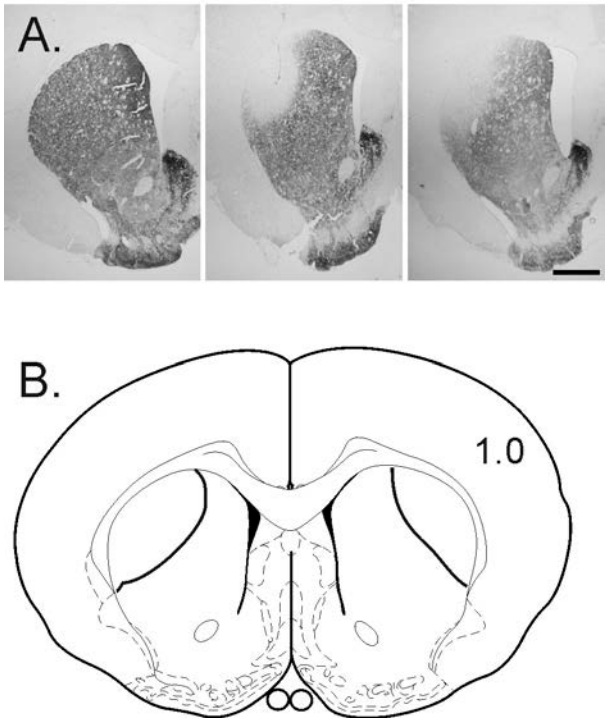


Figure 11. Dorsolateral striatal lesions. (**A**) Representative photomicrographs of TH-IHC at the level of the DLS (~1.0mm AP from bregma) in one hemisphere. Compared to sham (vehicle-injected; left), 6-OHDA reduced staining in the DLS at 21 days (middle) and 60 days (right) post treatment. Scale bar = 1mm. (**B**) Bilateral illustration of the extent and location of 6-OHDA-induced lesions 21 days after injection. For the six rats killed at this time, the tissue section which were targeted during surgery (1.0mm anterior to bregma) were analyzed by two observers. Each independently outlined the TH-like staining for the section for each rat. The outer most borders delineated by lack of staining was determined. Illustrated are the outlines for the largest lesion area from both observers (neuroanatomical plates modified from Paxinos and Watson (Paxinos and Watson 1998). The borders of the lesion after 60 days were less discrete (see A, far right); but in general, the lesion size was similar to that seen at 21 days.

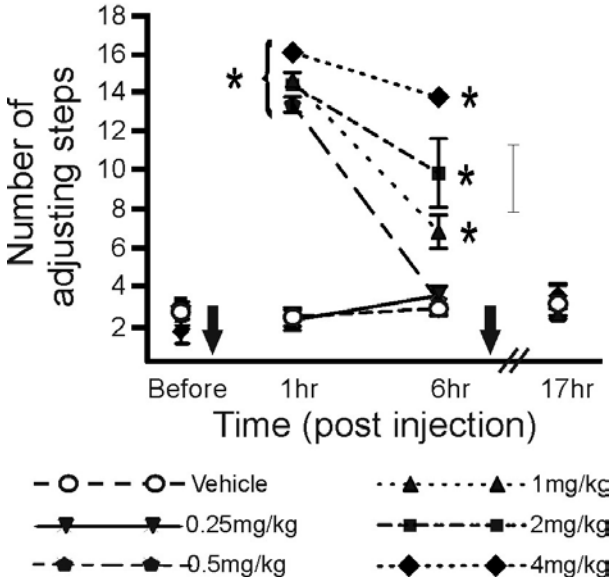


Figure 12. Motor deficits produced by intra-dorsolateral striatal injections of 6-OHDA are reversed by pramipexole. Illustrated is adjusting stepping from the left forelimb in the abduction direction for PD-like rats. Approximately 40 days after the lesion surgery, rats underwent a series of weekly step tests. Pre-PPX deficits (Before) were obtained immediately prior to the PPX injection. PPX reversed these stepping deficits in a dose-dependent manner. PPX significantly increased the number of adjusting steps with 0.5, 1, 2 and 4mg/kg at 1hr, while at 6hr this increase was only seen with 1, 2 and 4mg/kg. The number of adjusting steps returned to pre-treatment levels 17hr after injection. No change from before injection was seen after an injection with vehicle or 0.25mg/kg of (±)PPX. *Post hoc* Newman Keuls: *, *vs.* before (±)PPX injection. Arrows indicate times of (±)PPX injection.

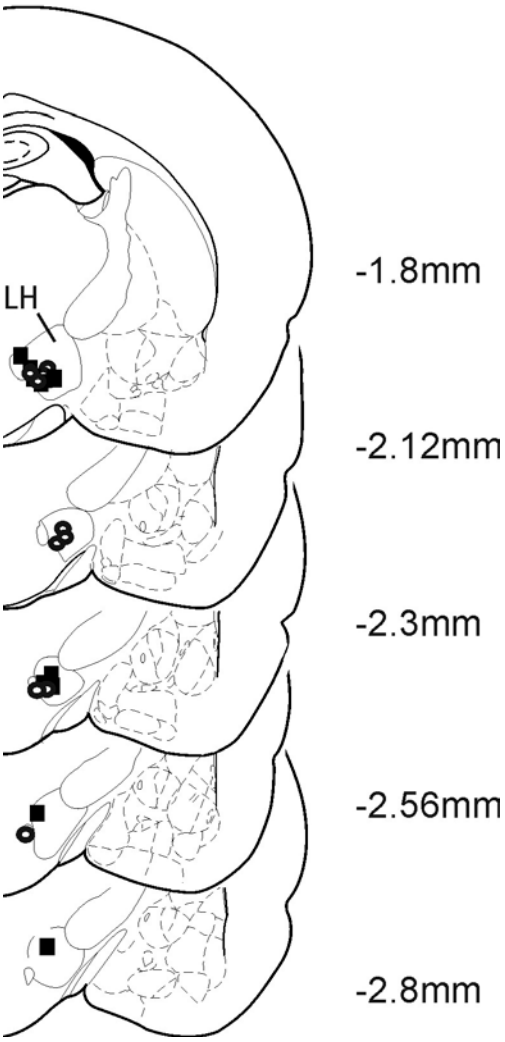

LH
-1.8mm
-2.12mm
-2.3mm
-2.56mm
-2.8mm

Figure 13. Electrode placement for intracranial self-stimulation (ICSS). Illustration of the stimulation electrode tip location within the lateral hypothalamus (LH) for 6-OHDA-treated (open circles, n=11) and sham (closed squares, n=10) rats that completed the probability discounting paradigm. Neuroanatomical plates were modified from Paxinos and Watson (Paxinos and Watson 1998) and numbers indicate the distance in mm from bregma. Note that the LH regions stimulated were similar for both groups of rats.

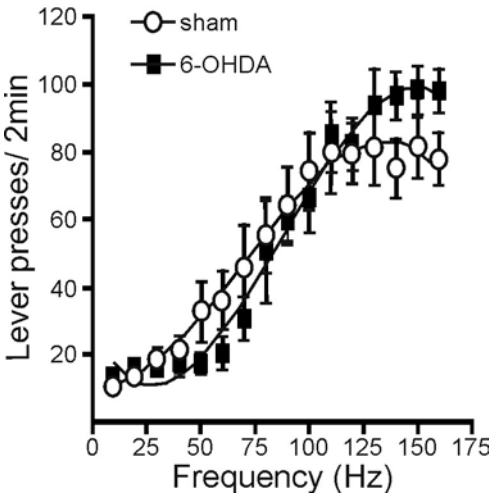


Figure 14. ICSS rate-frequency functions: comparisons between 6-OHDA-treated and sham rats. The relationship between ICSS lever pressing rate and stimulation frequency was similar for 6-OHDA-treated (n=11) and sham (n=10) rats. Shown are the group means±SEM from stable curves generated by each rat. Plots are drawn as a third order polynomial to visualize Emax and threshold.

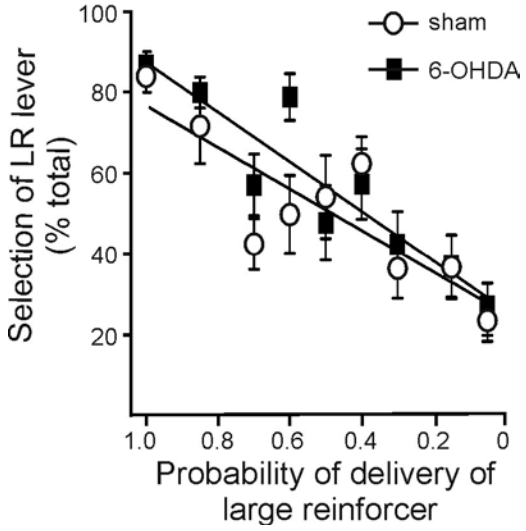


Figure 15. Acquisition of the probability discounting task. During Phase 6, 6-OHDA-treated (n=11) and sham (n=10) rats acquired the probability discounting task during the first training session. Illustrated are the group means±SEM for the percent selection of the large/risky (LR) lever (i.e., free-choice ratio) *vs.* the probability that the large reinforcer was delivered *for the first discounting session*. The plot is drawn as a linear regression.

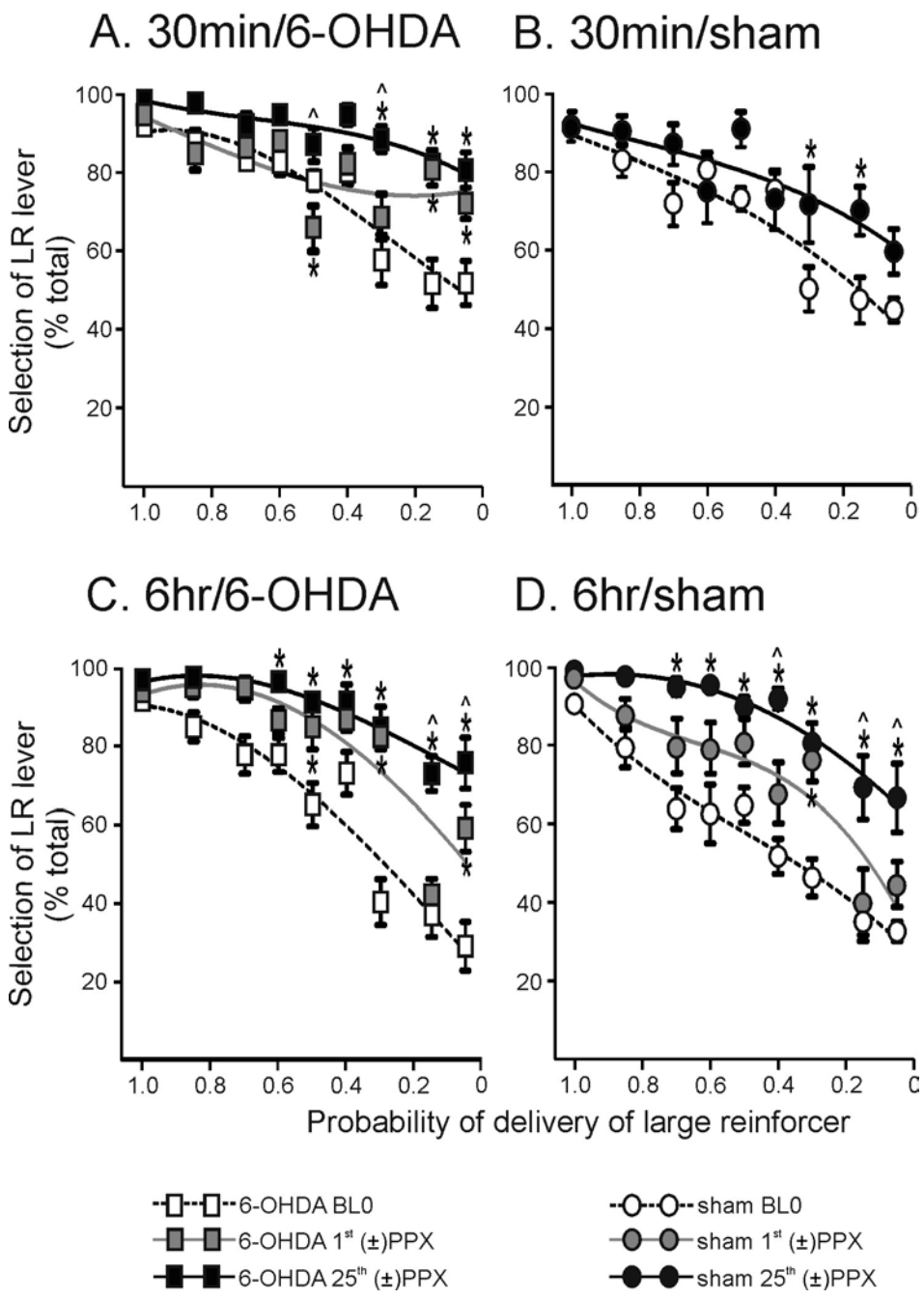

A. 30min/6-OHDA
B. 30min/sham
C. 6hr/6-OHDA
D. 6hr/sham
Selection of LR lever (% total)
Probability of delivery of large reinforcer
6-OHDA BL0
6-OHDA 1st (±)PPX
6-OHDA 25th (±)PPX
sham BL0
sham 1st (±)PPX
sham 25th (±)PPX

Figure 16. Pramipexole increased probability discounting. In Phase 7, 6-OHDA-treated (n=11) and sham (n=10) rats received 2mg/kg (±)PPX ip twice a day for 13 days for a total of 26 injections. Discounting sessions were conducted 30min and 6hr after the morning (AM) injection, on the first and every third day after initiating the treatment. Data from these two sessions were compared to the pretreatment baseline (BL0) for the respective time periods. PPX increased discounting in 6-OHDA-treated rats tested after the first PPX treatment and the 25th (±)PPX treatment at both (**A**) 30min and (**C**) 6hr post injection. Similar increases in discounting were seen in sham rats (**B**) 30min and (**D**) 6hr after PPX treatment. Shown is the percent selection of the large/risky (LR) lever (i.e., free-choice ratio) *vs.* the probability that the large reinforcer was delivered. *Post hoc* Newman Keuls: *, *vs.* BL; #, *vs.* 1st injection.

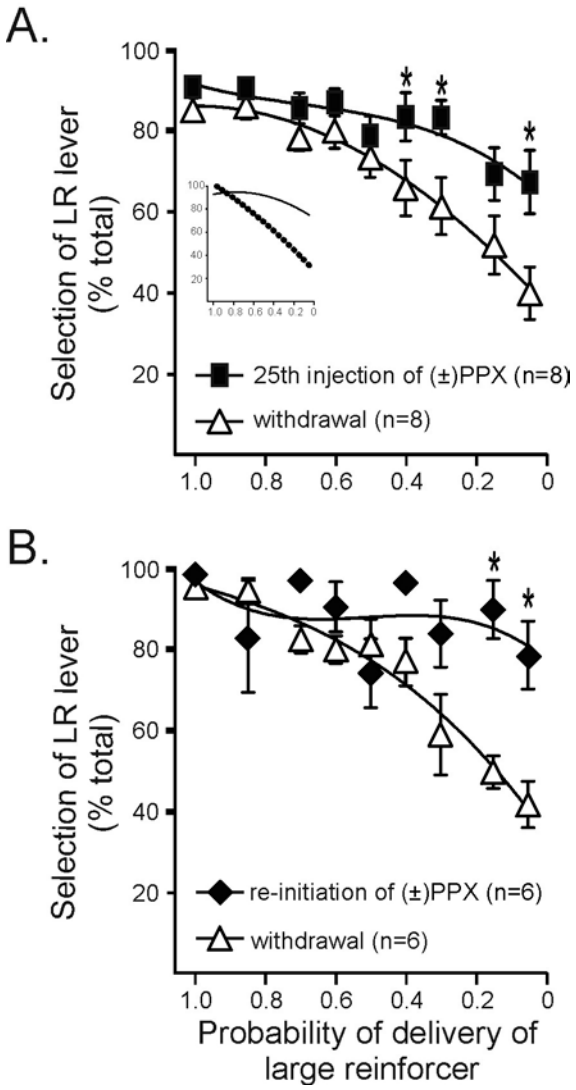

A.
Selection of LR lever (% total)
100
80
60
40
20
25th injection of (±)PPX (n=8)
withdrawal (n=8)
1.0 0.8 0.6 0.4 0.2 0
B.
Selection of LR lever (% total)
re-initiation of (±)PPX (n=6)
withdrawal (n=6)
Probability of delivery of large reinforcer

Figure 17. Withdrawal from pramipexole decreased probabilistic discounting while re-initiation of pramipexole reinstated the increase in discounting. Shown is the percent selection for the large/risky (LR) lever (i.e., free-choice ratio) *vs.* the probability that the large reinforcer was delivered. **(A)** Phase 8; PPX treatments were terminated. Illustrated are data from 6-OHDA-treated rats (n=8). Discounting measured on days 12 and 15 of withdrawal were averaged for each rat, and group data were compared to discounting obtained 30min after the 25th PPX injection. Inset illustrates data from sham rats (n=3); smooth line indicates 25th injection of (±)PPX and dotted line indicates withdrawal phase. **(B)** Phase 9; ±PPX treatment was re-initiated in a subset of withdrawn 6-OHDA-treated rats (n=6). Rats received 2mg/kg (±)PPX ip twice a day for seven days for a total of 14 injections. Discounting measured on the last two withdrawal days was averaged for each rat, and group data were compared to discounting data collected after the 13th (±)PPX injection during re-initiation. *Post hoc* Newman-Keuls: *, *vs*. withdrawal.

CHAPTER V

PRAMIPEXOLE-INDUCED CHANGES IN VENTRAL PALLIDAL NEURONAL FIRING: EVALUATIONS IN 6-OHDA-INDUCED PARKINSONIAN-LIKE BRAIN STATES OR FOLLOWING REPEATED TREATMENT WITH PRAMIPEXOLE

Abstract

Pramipexole (PPX) is a D3-preferring D2/D3 dopamine (DA) receptor (D2/D3R) agonist used for therapy in neurological disorders, including Parkinson's disease (PD). Its clinical use has been linked to impulse control disorders (ICDs) in some patients. In laboratory rats, PPX can increase measures of impulsivity and enhance the motivational salience of reward-related cues. The ventral pallidum (VP) encodes and regulates salience attribution and exhibits high expression of D2/D3Rs. In the current study, we determined the effects of systemic (-)PPX (the active enantiomer) on VP neuronal activity. A range of doses previously shown to alter reward-related behaviors were tested. The expression level of D2/D3Rs reportedly changes following partial and/or severe degeneration of dopaminergic neurons in the nigrostriatal system as well as after repeated treatment with DA agonists. Thus, we investigated whether such chronic conditions altered PPX-induced responding by VP neurons. To do so, we recorded the extracellular spiking activity of VP neurons in chloral hydrate-anesthetized rats in controls, following 6-OHDA-induced lesions of the dorsolateral striatum (DLS) in both

hemispheres, following a 6-OHDA-induced lesion of the medial forebrain bundle (MFB) in one hemisphere, or following14 days of twice daily injections with 2mg/kg (±)PPX (a racemic mixture of the drug that is equivalent to 1mg/kg of the active form). We determined that basal neuronal activity did not differ among the controls and the three treatment groups. However, in response to acute intravenous injections of PPX, the potency (i.e.,ED_{50}) was enhanced in rats treated chronically with PPX; the maximal effect (E_{max}) of PPX was not altered. Compared to controls, potency and maximal effect were not altered in rats with lesions to the DLS nor the MFB. Antagonism of the PPX-induced effects was achieved with PG-01037, a D3R-preferring antagonist, in the majority of VP neurons that responded to 300µg/kg (-)PPX. The findings indicate that the VP is engaged by doses of PPX that alter reward-related behaviors and that D3Rs play a role in such responses. Moreover, a chronic treatment regimen with PPX, that our lab previously demonstrated can increase risk-taking behavior, increased the potency of PPX to alter VP neuronal firing rate. Possible implications for the role of the VP in PPX-induced ICDs are discussed.

Introduction

Pramipexole (PPX) is a full dopamine (DA) agonist that is FDA-approved for therapy of motor impairments in Parkinson's disease (PD). These motor impairments result from a decrease in striatal DA transmission in the nigrostriatal system. In early stage PD, the DA projections that comprise the mesolimbic pathway are left relatively intact (Bernheimer et al., 1973; Kish et al., 1988). PPX acts on the D2 receptor family and has a slight preference for the D3 receptor subtype (D3R) over the D2 receptor

subtype (D2R) (~30-fold selective for D3R over D2R (Collins et al., 2007). Whereas D2Rs are highly expressed throughout the nigrostriatal and mesolimbic systems, D3R are relatively less abundant and restricted more to the mesolimbic regions (Bouthenet et al., 1991). At the higher doses administered to patients, PPX acts on both D2R and D3Rs. The motoric improvements are likely due to a normalization of nigrostriatal activity; however, D2/D3Rs in the intact mesolimbic system are also being activated. It is now well documented that a subset of PPX-treated patients develop impulse control disorders (ICDs) that includes behavioral addictions such as pathological gambling, hypersexuality, impulsive shopping and overeating (Weintraub et al., 2010). It is thought that these individuals engage in these maladaptive behaviors because of the influence of PPX on the intact mesolimbic system (Cools et al., 2006).

The ventral pallidum (VP) is a brain structure located at an interface between the mesolimbic system and the nigrostriatal system (see Fig. 3 for a circuit diagram). The VP integrates efferent projections from the nucleus accumbens (NA; Groenewegen et al., 1993; Nauta et al., 1978; Chrobak and Napier, 1993)), amygdala (AMG; (Krettek and Price, 1978; Bayer et al., 2007; Leonard and Scott, 1971; Mitrovic and Napier, 1998; Maslowski-Cobuzzi and Napier, 1994)), prefrontal cortex (PFC; (Delgado-Martinez and Vives, 1993; Sesack et al., 1989)), subthalamic nucleus (STN; (Turner et al., 2001; Groenewegen and Berendse, 1990), VTA and SN pars compacta (Maslowski-Cobuzzi and Napier, 1994; Mitrovic and Napier, 2002; Klitenick et al., 1992). The VP projects to the NA (Churchill and Kalivas, 1994; Hakan et al., 1992), STN (Maurice et al., 1997; Bell et al., 1995), PFC (Sesack et al., 1989), medial dorsal thalamus (Churchill et al., 1996; O'Donnell et al., 1997), VTA (Groenewegen et al., 1993; Kalivas et al., 1993),

substantia nigra pars reticulata (SNpr; (Maurice et al., 1997)) and brainstem targets including the pedunculopontine nucleus (PPN; (Tsai et al., 1989)). Based on these inputs and outputs of the VP, it is in a critical position to integrate limbic-processed reward information and influence final motor activation (Mogenson et al., 1980).

Behavioral and electrophysiological studies reveal a direct involvement of the VP in reward-related behavior. VP firing can track rewards and the incentive salience of reward-predictive cues so that the cues become "wanted" (Tindell et al., 2005; Tindell et al., 2004). VP neurons also encode expected reward values (Tachibana and Hikosaka, 2012). The VP is also involved in reward based associative learning (Dallimore et al., 2006; Mickiewicz et al., 2009; Gong et al., 1996) and reward seeking behaviors (Tang et al., 2005; McFarland and Kalivas, 2001). Activation of the VP was detected during a human functional MRI study in which there was increased motivational behavior in response to cues that predicted the potential gain of a large quantity of money (Pessiglione et al., 2007). Our lab has reported that PPX can induce reward-mediated associative learning as measured in a conditioned place preference paradigm (Riddle et al., 2012). Others have shown in rats that PPX can increase salience of cues previously associated with cocaine (Collins et al., 2011). Thus, the ability for PPX to increase the motivational salience of reward predicting cues may, at least in part, be due to VP activity.

Here, we examined the consequence of intravenously (iv) injected PPX (1-3000µg/kg) on VP neuronal firing rate, using single cell extracellular electrophysiological recording techniques in chloral hydrate anesthetized rats. This range of doses includes those that alter reward-mediated behavior (Riddle et al., 2012; Caine et

al., 1997; Collins et al., 2011). We considered that if systemic PPX does change the firing rate of VP neurons, this will demonstrate that the VP is engaged by PPX and would support a role for the VP in reward-motivated behavioral outcomes of PPX therapy. The range of doses includes the lower, more D3R-selective doses, as well as higher doses that will activate D2Rs (Collins et al., 2005; Collins et al., 2008; Collins et al., 2007). The VP (Stanwood et al., 2000a), as well as the related limbic circuitry (Bouthenet et al., 1991), express a moderate to high level of D3Rs. D3R are located presynaptically on dopaminergic midbrain neurons where they function as autoreceptors (Meller et al., 1993; Gobert et al., 1995). Lesions studies provide evidence that D3Rs are also expressed on cell bodies of the NA (Stanwood et al., 2000a). D3Rs have gained much attention in the areas of reward and impulsivity (see (Heidbreder and Newman, 2010). It has been speculated that PPX-induced activation of these receptors in particular, might be driving the ICDs (Dodd et al., 2005; Fan et al., 2009). Indeed, in rodent studies, activation of D3Rs enhances the motivational salience of rewards and can strongly modulate the influence of environmental stimuli on seeking behavior (Orio et al., 2010; Higley et al., 2011; Gilbert et al., 2005). To help determine influence of D3Rs on PPX-induced changes in VP neuronal firing rate, we took advantage of the D3R-preferring doses of PPX to ascertain if the responses could be blocked by a D3R-preferring antagonist, PG01037 (133-fold selectivity for D3R over D2R; (Grundt et al., 2005)).

Given that DA agonist-induced ICDs occur in PD patients, another aim of this study was to determine if the effects of PPX on the firing rate of VP neurons are altered in the parkinsonian brain state. We hypothesized a change in PPX-induced effects because the VP has both direct and indirect connections with the basal ganglia, and the

VP is altered in PD-like brain states (Turner et al., 2002). As PPX is prescribed to patients throughout the course of PD, we chose to study both an early and late stage model of PD. We used a partial lesion of the dorsolateral striatum (DLS) to emulate important features of early stage PD, which include region specificity and motor impairments. For example, early in the disease reductions in DA are restricted to the putamen (i.e., part of the dorsal striatum), with the ventral striatum being relatively spared (Bernheimer et al., 1973; Kish et al., 1988). In addition, once putamen DA is decreased by 50-80%, motor impairment start to occur (Bernheimer et al., 1973; Guttman et al., 1997; Morrish et al., 1998). In the rat brain, the lateral striatum is thought to be homologous to the human putamen (Deumens et al., 2002). In particular, the DLS receives projections from the sensorimotor cortex that are responsible for forepaw motor function (Deumens et al., 2002; Chang et al., 1999; Carli et al., 1985). We and others have demonstrated that 6-OHDA targeted to the DLS produces persistent and stable deficits in the adjusting forelimb stepping task (Olsson et al., 1995) up to two months post lesion (Rokosik and Napier, 2012; Riddle et al., 2012; Chang et al., 1999). The current electrophysiological studies were conducted 20-30 days post DLS lesion. This time frame was chosen because it corresponds to when the lesion of the nigrostriatal pathway (at both the terminals and cell bodies) stabilizes (Blandini et al., 2007) and motor deficits are present (Rokosik and Napier, 2012).

To study the later stages of PD we used a unilateral 6-OHDA-induced lesion targeted to the medial forebrain bundle (MFB). This type of lesion produces near complete destruction of the ascending dopaminergic system in the injected hemisphere (Heidenreich et al., 2004; Ungerstedt, 1968) and also produces motor deficits

contralateral to the lesion, as can be measured in the forelimb stepping task (Chang et al., 1999). In late stages of PD, both striata are affected. However, although a bilateral lesion would be optimal to study for late stage PD, such a lesion can interfere with the ability for the rat to eat and drink and thus survive (Ungerstedt, 1971; Ljungberg and Ungerstedt, 1976). Therefore, to circumvent this issue, we chose to use a unilateral lesion. Our electrophysiological studies were conducted 10-16 days post MFB lesion. This time frame was chosen because it corresponds to when the lesion of the nigrostriatal pathway (both fiber degeneration and cell death) stabilizes (Jeon et al., 1995) and forepaw motor deficits are present (Chang et al., 1999). The time frame was also chosen based on electrophysiological data that demonstrate alterations in the neurocircuitry under study. For example, increases in firing rate and oscillatory activity are evident in the STN within this time frame (Parr-Brownlie et al., 2007). Additionally, our lab has demonstrated that 6-OHDA-induced lesions to the SN that produce near total depletion of DA in the ascending dopaminergic projections enhance the effects of NMDA receptor activation in VP neurons (Turner et al., 2002). This supports the idea that sensitivity in VP neuronal firing can occur after chronic depletion of DA in the ascending dopaminergic projections.

PPX-induced ICDs are seen in PD, as well as other neuropathologies such as restless leg syndrome (Driver-Dunckley et al. 2007), fibromyalgia (Holman 2009), and bipolar disorders (Strejilevich et al. 2011). In these cases, ICDs develop during the chronic use of PPX. This implies that over time adaptations that occur in an attempt to maintain homeostasis in the brain, are contributing to the ability of PPX to induce ICDs. Our lab recently reported that acute and chronic treatment (14 days twice daily injections)

with 2mg/kg, (±)PPX (a racemic mixture equal to 1mg/kg of the active racimer) enhances probability discounting, an index of risk-taking, in both controls and a rodent model of early stage PD (Rokosik and Napier, 2012). This behavior was maintained three days after the last PPX injection. In the current study, we investigated whether PPX-induced changes in VP neuronal firing in an intact rat was altered following the same chronic drug treatment and withdrawal time period that produced the increase in risk-taking. As described below, adaptations occur in the PD-like brain state, as well as during chronic PPX treatment. Therefore, to best isolate the contribution of PPX-induced plasticity, the chronic PPX treatment studies were done in control rats only.

In the current study, measured outcomes of the PPX-induced effects for all treatment conditions included the *efficacy* of PPX and *potency* of PPX to alter firing rate of VP neurons. Efficacy is a measure of the maximal effect (E_{max}) of the agonist. Potency is a measure of how much drug is required to elicit a given response. It is typically measured as the dose of the drug that gives 50% of the maximal response (ED_{50}). Changes in the number of receptors can influence the efficacy and/or potency of a drug (Kramer et al., 2011). We hypothesized that E_{max} and ED_{50} of PPX would be altered in rats with a 6-OHDA-induced lesion to the DLS, rats with a 6-OHDA-induced lesion to the MFB, and rats treated chronically with PPX. This hypothesis was based on the following: Regarding the PD state **(i)** dopaminergic cell loss in PD and subsequent denervation to output structures leads to an increase in D2Rs and a decrease in D3Rs (Rinne et al., 1990; Brooks et al., 1992; Ryoo et al., 1998). **(ii)** In rats with a unilateral 6-OHDA-induced lesion to the MFB, D3R expression and mRNA in the NA and SN decrease on the lesion side only (Bordet et al., 1997; Stanwood et al., 2000a; Levesque et

al., 1995) whereas D2R expression and mRNA increases in these areas (Levesque et al., 1995; Stanwood et al., 2000a). Studies have yet to investigate changes in D2R and D3R expression following 6-OHDA-induced DLS lesions. Regarding chronic agonist treatment **(iii)** sustained increase in dopaminergic transmission typically downregulates D2Rs but upregulates D3Rs. For example, in rats a fourteen day treatment with D2/D3 agonists, 7-OH-DPAT or quinipirole, increases expression of D3Rs in the VP and SN and decreases D2R in the VP, SN and NA (Stanwood et al., 2000b). **(iv)** Rats treated for 14 days with PPX (0.3 and 1mg/kg) show increase expression of D3Rs in the NA (D2R expression was not studied; Maj et al., 2000; Tokunaga et al., 2012).

In summary, the overall aim of this paper was to determine the effects of acute systemic PPX on VP neuronal activity and the involvement of D3Rs in the measured responses. Furthermore, we wanted to determine if the effects of PPX were altered in two different animal models of PD as well as in rats that were treated with a dosing regimen of chronic PPX known to produce an increase in risk-taking behaviors. The following hypotheses were made for this study: **(i)** VP neurons will show a change in firing rate in response to systemically administered PPX. **(ii)** The D3R preferring antagonist, PG01037, will attenuate the PPX induced alterations in VP neuronal firing. **(iii)** Compared to controls, the potency and maximal effect of PPX will be altered in both rodent models of PD, as well as in rats treated chronically with PPX.

Materials and methods

Male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 225–274 g upon arrival were housed in pairs under the environmentally controlled conditions of the local

vivarium (7:00 AM/7:00 PM light/dark cycle, temperature maintained at 23–25 ◦C) with *ad libitum* access to rat chow and water. All rats were handled according to established procedures in the Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, DC). Experiments were carried out at Loyola University Medical Center and Rush University Medical Center. Specific protocols were approved by the Institutional Animal Care and Use Committee at each university.

Treatment Drugs

To induce dopaminergic lesions, 6-hydroxydopamine-HBr (6-OHDA; Sigma-Aldrich, St Louis, MO) was dissolved in 0.2% ascorbic acid in a sterile saline solution (pH=5.0) and infused into the MFB at a dose of 12µg per 4µl per side (as the salt) or infused into the striatum at a dose of 7.5 µg per 2 µl per side. Rats were given a 30-min intraperitoneal (ip) injection pretreatment of desipramine-HCl (DMI; Sigma-Aldrich; 25-30 mg/kg, as the salt, dissolved in sterile water) and pargyline (Sigma-Aldrich; 50mg/kg, as the salt, dissolved in 0.9% sterile saline) to aid 6-OHDA in lesioning the dopaminergic system. Pramipexole (synthesized as the racemic mixture; Daya Drug Discoveries, Hazelwood, MO) (±PPX) was dissolved in saline and given ip as 2 mg/kg for chronic treatment in a subset of rats. For all electrophysiological experiments, (-)PPX (the active S(-) enantiomer; Tocris, Ellisville, MO) was given at doses (0.001-3.0mg/kg, as the salt and equal to 1-3000µg/kg) that were administered intravenously (iv) in a series of cumulative and divided doses. PG01037 was generously provided by Dr. Amy H. Newman (Medicinal Chemistry Section, National Institute on Drug Abuse, Baltimore, MD). It was dissolved in 2% tween-80 (Sigma-Aldrich) and given at three doses (3, 10,

and 30mg/kg, as the salt) that were administered iv in a series of increasing cumulative doses.

Surgical procedures for the medial forebrain bundle and dorsolateral striatal 6-hydroxydopamine-induced lesions

To stereotaxically lesion the MFB, rats were anesthetized with an ip injection of sodium pentobarbital (50 mg/kg/ml; Sigma-Aldrich) or continuous isofluorane inhalation. The head was placed in a stereotaxic frame (David Kopf, Tujunga, CA) with the nose piece set at 3.3mm below the horizontal. For 6-OHDA injections into the MFB, a 33-gauge, bilateral injector was lowered to the (4.2 mm anterior to lambda, ±1.6 mm from midline, 8.5 mm ventral from skull). Following a 30min pretreatment with DMI and pargyline, 6-OHDA was injected unilaterally at a rate of 0.5 µl/min for four minutes. The ascorbic acid vehicle was similarly infused in the contralateral hemisphere. The injectors were left in place for an additional min (to allow the solution to diffuse away from the tip), and upon removal the skull holes were filled with bone wax. The procedure for the lesions of the DLS was similar to the MFB lesion. In brief, rats were anesthetized with continuous isofluorane inhalation and given a 30min DMI pretreatment (25mg/kg, ip). A 33-gauge, bilateral injector was lowered to the DLS (1.0mm anterior to bregma, 3.4mm lateral from midline, 4.7mm ventral from skull) and 6-OHDA was injected at a rate of 0.2 µl/min, for a total of ten minutes. Sham controls were similarly injected with the ascorbic acid vehicle.

Rats with a unilateral MFB lesion were prepared for electrophysiological studies 10-17 days post lesion. Rats with a bilateral DLS lesion were prepared for electrophysiological studies 20-30 days post lesion.

Chronic DA agonist treatment in intact rats

Rats received 2mg/kg ±PPX, ip, twice daily for 14 days. Rats were prepared for electrophysiological recordings 2-4 days after the last drug injection.

Extracellular electrophysiology

Surgical Preparation

Rats were anesthetized with chloral hydrate (400mg/kg, ip) and the lateral tail vein was cannulated to allow for iv administration of treatment drugs and supplements of chloral hydrate. The rat was placed in a stereotaxic frame with the nose piece set at 3.3mm below the horizontal. To access the VP, the skull was exposed and a burr hole was drilled through the skull 0.2-0.6 mm posterior to bregma, 2.0-3.0 mm lateral to the midline. Body temperature was maintained at 36º C throughout the experiment.

Extracellular recording

Action potentials from VP neurons were recorded extracellularly though a single barrel micropipette pulled from 2.0 mm O.D. glass tubing (A-M Systems, Inc., Everett, WA) with a vertical puller (Narishige PE-2, Tokyo, Japan). The tip was broken back to a diameter of approximately 2 µm and the electrode was filled with a 2% pontamine sky blue/0.5 M sodium acetate solution. The impedance of these electrodes was 3-8 MΩ, as measured in 0.9% saline at 165 Hz with a microelectrode tester (Winston Electronics Company, Millbrae, CA). To record from VP neurons this glass microelectrode was lowered through the burr hole with a hydraulic microdrive (Trent Wells, South Gate, CA) to a depth of 7.5-8.5 mm ventral to dura.

Electrical signals recorded by the electrode were passed through a high-impedance amplifier (Fintronics, Inc., Orange, CT), filtered (200-2000 Hz cutoffs) and monitored on an oscilloscope (Tektronix, Inc., Beaverton, OR) and an audiomonitor (Grass Instruments, Quincy, MA). The signals were relayed to a window discriminator (Fintronics, Inc.) with the digital output representing action potentials from single, spontaneously active neurons. The output was recorded by a computer that, with the aid of custom software, displayed rate histograms and stored all data for future statistical analysis. The action potentials were characterized by waveform (i.e., biphasic with an initially negative voltage deflection or triphasic with an initially positive voltage deflection), amplitude (peak to peak) and, duration (μsec). Neuronal firing was characterized by rate and pattern (i.e., interspike interval (ISI)). To be included in these evaluations, the ratio of the action potential amplitude to background "noise" had to be at least 4:1. Data were collected from one neuron per rat.

After recording a minimum of five minutes of stable neuronal firing activity (< 20% variability), rats received an iv injection of 0.1 ml saline followed by eight injections of (-)PPX (0.001-3.0mg/kg given in a cumulative fashion). Each iv injection was given at two min intervals. The saline injection was given as a control for volume injection; a saline effect was defined as >20% change from baseline. If, on a rare occasion, a saline effect was obtained, data from that neuron were excluded from further analysis.

A separate group of intact rats received a single iv injection of saline (0.1ml) followed by injection of 0.3mg/kg (-)PPX. Subsequently, rats were administered three doses of PG01037 (3, 10, and 30mg/kg iv given in a cumulative fashion). Saline and PPX were given in two min intervals, whereas PG01037 was given in 5 min intervals.

At the completion of all the experiments, an anionic current was passed through the microelectrode to deposit pontamine sky blue, thus marking the location of the tip of the microelectrode. Rats were then overdosed with chloral hydrate and decapitated. Brains were extracted and placed in a10% buffered formalin solution (Fisher, Kalamazoo, MI) for 48hr, and then stored in a 30% sucrose/0.4M phosphate buffered solution. For histological evaluations, frozen brains were sliced in 40 mm coronal sections. The location of markers for the tip and track of the electrodes were examined and agreed upon by two people. A randomly selected subset of rats was used for tyrosine hydroxylase (TH; the rate limiting synthetic enzyme for DA) immunohistochemistry (IHC) evaluations of the 6-OHDA-induced lesions. Tissue was processed for TH-IHC using the protocol previously employed (Rokosik and Napier, 2012).

Data Analysis

Neuronal firing rate following injections was transformed to a percent of pretreatment control (i.e., baseline), determined by comparison of the firing rates averaged over the last 60 sec interval of baseline (considered 100%) with that observed during the last 60 sec interval following each injection. A "response" to PPX was considered to have occurred if firing rate changed from baseline by at least 20% during two applications of the agonist with the inclusion of the 300µg/kg dose. The 300µg/kg dose was chosen because it falls within the range of selectivity for the D2/D3R (which allows us to capture the influence of both D2 and D3Rs) is near the maximal effect (which allows us to capture a near complete dose response, but does not extend into the plateau where physiological limits may mask a dose-related response). We categorized

responses based on the ability of PPX to produce rate-enhancements, rate-suppression, biphasic responses, or no change. For cells that demonstrated a "response", a third order polynomial was fit to the data and a regression analysis was performed (GraphPad Prism, La Jolla, CA). For regressions that demonstrated an $r^2 \geq 0.7$, cumulative log dose-response curves were generated to determine the maximal effect (E_{max}) of the agonist and the dose that produced 50% of the maximal effect (ED_{50}; GraphPad Prism). In order to statistically assess the single point parameters, E_{max} and ED_{50} were determined for each neuron, and the geometric mean of each measure was calculated for the treatment group.

To determine if the maximal effect and/or the potency of PPX were altered by 6-OHDA-induced lesions or repeated treatment with PPX, direct comparisons in dose-response relationships among the treatment groups were made. To aid in this analysis, we standardized PPX effects as a percent of baseline. Both rate enhancements and suppressions were observed; however, there was no difference between the geometric E_{max} and ED_{50} of PPX-induced increase and decreased rate profiles (data not shown). This finding is consistent with the idea that rate increases and decreases reflect similar pharmacological mechanisms at the drug/receptor level, and the different firing rate outcomes reflect circuit-mediated direct and indirect effects. Thus, for purposes of analyzing shifts in the dose-response relationship as an index of changes in drug/receptor interactions, we evaluated PPX-induced responses as an absolute change from baseline. The mean values of E_{max} and ED_{50} in the various treatment groups were compared using either an ANOVA or Student's *t*-test. If the calculated E_{max} or ED_{50} was more than 2 standard deviations away from the mean, it was considered to be an outlier (and thus an erroneous estimate of the event) and the neuron was excluded from analysis. Using these

criteria, no more than one cell was omitted per treatment condition. Firing patterns were quantified by a mean to mode ratio ISI using customized software. A Student's *t*-test or ANOVA were used to compare neuronal firing characteristics (i.e., firing rate and pattern), electrophysiological profiles and the pharmacological assessments across control and treatment groups. A Student's *t*-test was also used to determine if the magnitude of the PPX response differed when given as a cumulative dose or single injection. A one way repeated measures (rm)ANOVA was used to compare the ability of different doses of PG01037 to antagonize the single PPX dose effect. If ANOVA was significant, Newman-Keuls pairwise *post hoc* evaluations were then used when appropriate. Categorical comparisons were accomplished using Pearson's *Chi*-square (χ^2) test of association or a Fisher's exact test. Significance of all statistical evaluations was set at $p \leq 0.05$.

Results

Tyrosine hydroxylase immunohistochemistry

The striatum ipsilateral to a vehicle injection into the MFB exhibited robust TH-IHC staining, while the striatum ipsilateral to MFB 6-OHDA injection into was essentially devoid of TH-IHC (Fig. 18A), similar to our prior work (Heidenreich et al., 2004; Muma et al., 2001). Also similar to our previous work, rats that received bilateral 6-OHDA injections into the DLS had reduced TH-IHC staining that was confined to the targeted area (Fig. 18B; Rokosik and Napier, 2012; Riddle et al., 2012).

Control groups for assessing effects of dopaminergic lesions or chronic PPX treatment on both basal and PPX-induced changes in the electrophysiological profile of ventral pallidal recordings

For the current study, we used the vehicle-DLS group as a control comparison for the 6-OHDA-DLS and 6-OHDA-MFB groups. This decision was supported by previous work from our laboratory that demonstrated injections of the 6-OHDA ascorbic acid vehicle to the MFB do not alter basal firing of VP neurons compared to intact controls (Heidenreich et al., 2004). Furthermore, as discussed below, we found that basal firing rate of neurons following vehicle injections into the DLS did not differ from intact controls (see Table 5). In the current study, we also used non-treated rats as a control for the chronic PPX treatment group. Our prior work also has verified that baseline electrophysiological characteristics of the VP are similar in rats that received repeated daily treatments of saline compared to untreated rats (McDaid et al., 2007).

Basal electrophysiological profile of ventral pallidal recordings and effects of dopaminergic lesions or chronic PPX treatment

Data were obtained from a total of 196 neurons located throughout the infracommisural and sublenticular VP (Fig. 19). Across the five treatment groups of rats (vehicle-DLS, 6-OHDA-DLS, 6-OHDA-MFB, non-treated, and chronic PPX), recording sites were distributed similarly. Two neuronal subpopulations have been identified within the VP (Zahm et al., 1985; Carsen et al., 1985). The majority of neurons are GABAergic and approximately 25% of VP neurons are cholinergic (Gritti et al., 1993). Prior extracellular studies of the basal forebrain of rats have suggested a correlation

between neuronal firing characteristics and neuronal subpopulations (Turner et al., 2001; Turner et al., 2002). To determine if subpopulations could be identified in the current study, and if so, whether the subpopulations would be differentially altered by either the 6-OHDA treatments and/or chronic PPX, we quantified several aspects of the action potential configuration and spiking activity.

Two distinct action potential profiles were observed; those with an initially negative-going, biphasic waveform and those with an initially positive-going, triphasic waveform. To assess differences in the amplitude and duration of these waveforms we pooled data from the non-treated and vehicle-DLS control groups (Table 4). Neurons that demonstrated a biphasic waveform produced action potentials with an amplitude of 459±15μV and a duration of 1.5 ± 0.02ms. Neurons that demonstrated a triphasic waveform produced action potentials with an amplitude of 76 6± 57μV and a duration of 1.3 ± 0.08ms. The amplitude and duration were significantly different between the biphasic and triphasic waveforms (see Table 4 for statistics). Consistent with our prior reports (Heidenreich et al., 2004; Turner et al., 2002), the two profile groups did not differ in firing rate or pattern (see Table 5 for statistics).

When comparing among the vehicle-DLS, 6-OHDA-DLS and 6-OHDA-MFB groups (shaded in green in Table 4 and 5) the amplitude of the biphasic waveform was significantly smaller in VP spikes from 6-OHDA-MFB rats as compared to the vehicle-DLS rats. When comparing among the non-treated and chronic PPX treatment groups (shaded in blue in Table 4 and 5), a larger amplitude of the triphasic waveform was obtained from neurons of the chronic PPX treatment group as compared to the non-treated control group. In this context, it is notable that our prior evaluations of VP action

potential profiles were not altered by 6-OHDA-induced lesions (Heidenreich et al., 2004; Turner et al., 2002). Note that amplitude is an action potential characteristic that can be related to proximity of the electrode tip to the axon hillock of the neuron being recorded (Purves, 1981), so care must be taken to assure the electrode is as close to the soma as possible. Moreover there should be a sufficient sample size to average out sampling variability of the amplitude. As differences in action potential characteristics (Table 4), but not spiking characteristics (Table 5) were found between neurons that demonstrated biphasic and triphasic waveforms, we pooled data collected from these recordings for all further assessments.

Procedural controls for studying the effects of acute PPX administration

The primary objective of this study was to determine if the VP neuronal responses to an acute treatment with PPX were altered by 6-OHDA-induced lesions to the DLS or MFB, or chronic treatment with PPX. This was accomplished by iv administration of multiple doses of PPX given in an escalating fashion to allow a complete dose-response analysis for each recorded VP neuron. Several control experiments were conducted to validate the dose-response protocol. In the first control, we verified that the multiple iv injections did not influence the firing rate of VP neurons across time. This was accomplished by administering nine saline vehicle injections given every two minutes that were in equal volume to that used to administer PPX) (see Fig. 20). This protocol verified that, in agreement with previous studies ((Heidenreich et al., 1995; Maslowski and Napier, 1991), the mechanical techniques of the injection or the increased volume of solution introduced to the circulatory system *via* the tail vein cannula did not alter VP

activity and that firing could be maintained within 20% of pretreatment baseline throughout the repeated infusion protocol. Thus, change in firing rate that was more than 20% from baseline was used as our criterion for a treatment effects. When a drug is repeatedly administered, factors such as tachyphylaxis (i.e., acute tolerance) may influence the measured outcome. In the second control experiment, we verified that PPX-induced changes in VP neuronal firing rates were similar following a single injection as compared to a the same dose achieved with multiple injections. For this comparison, we selected 300µg/kg (-)PPX, a dose that induced near maximal responses in the PPX multiple dose-response evaluations (see Table 6). A *Chi*-square analysis of the distribution of responses following either a cumulative divided dose or single injection indicated that the number of neurons in each category were not different. A *t*-test indicated that the magnitude of the average increase in firing rate induced by the single injection of PPX was not different from the response after the 300µg/kg cumulative dose, nor was the magnitude of the average suppression of firing rate. Thus, effects of the same dose of PPX were comparable with those observed after a single injection or as a divided cumulative dose.

The secondary objective of these studies was to determine if the D3R was involved in PPX-induced responses. To do so, we evaluated the ability of iv PG01037 to antagonize the effect of a single 300µg/kg iv injection of PPX; therefore, it was necessary to verify that the vehicle used for PG01037 was not able to alter responding to PPX. Accordingly, we determined that firing rate recorded for 15min following a PPX injection was stable and that three subsequent iv injections of PG01037 vehicle, given in five minutes intervals (the protocol used to test PG01037-mediated antagonism), did not

affect firing rate (see Fig. 21). Thus, any changes in firing rate that occurred after the administration of PG01037 were considered to be a consequence of the antagonist and not the wearing off of PPX-induced effects nor the effects of the vehicle solution.

Effects of PPX on VP neuronal firing rate in two models of Parkinson's disease

The effects of acute iv PPX on VP neuronal firing rate were assessed in rats with DLS injections of 6-OHDA or its vehicle (n=29 and n=24, respectively), and on VP neurons ipsilateral to unilateral 6-OHDA-injections into the MFB (n=37). A histogram example of a dose-related response (i.e., rate suppression) produced by cumulative doses of PPX is shown in Fig. 22 (shown also are the cumulative doses and timing intervals for this set of experiments). The number of 'responders' (i.e., those showing a (-)PPX-induced rate change $\geq$20% by 300µg/kg (-)PPX) *vs.* non-responders, and the distribution of responses among the three treatment groups, are summarized in Table 7. *Chi*-square analyses revealed no significant difference in the distribution of neuronal responses among the four categories (i.e., increase, decrease, biphasic or no change). These data suggest that neither partial lesions of striatal DA nor near complete depletion of the ascending a dopaminergic system influenced the ability 300µg/kg (-)PPX to alter firing of VP neurons.

Next we analyzed dose-response relationships between the 6-OHDA-MFB, 6-OHDA-DLS and vehicle-DLS groups (Fig. 23A). A two way repeated measures ANOVA revealed no effect of treatment group ($F_{(2,119)}$=0.01, p=0.99), an effect of dose ($F_{(7,96937)}$=20.29, p<0.001), and no interaction ($F_{(14,2580)}$=0.27, p=0.99). This indicates there was a dose related response to (±)PPX, but a lesion to the DLS or MFB did not

affect this response. To determine if either 6-OHDA-induced lesions altered PPX-induced responding in terms of efficacy and/or potency, a one way ANOVA was used to compare E_{max} and ED_{50} among the three groups. No difference was obtained for E_{max} (Fig. 23B), but there was a difference in ED_{50} among the groups (Fig. 23C). A *post hoc* Newman-Keuls analysis of the ED_{50} revealed that neither the 6-OHDA-DLS or 6-OHDA-MFB group was different from the vehicle-DLS group; however, there was a difference between the 6-OHDA-DLS and 6-OHDA-MFB groups. It is noteworthy that there does appear to be a trend towards a greater potency of PPX (i.e., a lower ED_{50}) in the 6-OHDA-MFB group compared to vehicle-DLS.

To determine if the sensitivity of VP neurons to respond to D3R-selective doses or D2/D3 selective doses of PPX was altered in the 6-OHDA-DLS and/or 6-OHDA-MFB groups, we determined the percent of neurons in each group that showed a response ($\geq$20% change in firing rate) at each PPX dose tested. All neurons tested were included. To assure that the response was not due to chance, the response requirement had to be met for at least two consecutive doses. Fig. 24 is a histogram of the results. Separate *Chi*-square analyses conducted at each dose revealed that compared to the vehicle-DLS group and the 6-OHDA-DLS group, there were significantly more neurons in the 6-OHDA-MFB group that responded to higher doses (i.e., 300 and 1000µg/kg). Taken together, these data suggest that partial lesions of the nigrostriatal pathway do not alter the potency of PPX to alter VP neuronal firing, but a more complete lesion of the ascending dopaminergic pathway may enhance the potency of PPX and this may be reflected in an enhanced sensitivity to D2R stimulation compared to D3R stimulation.

Effects of PPX on VP neuronal firing rate in rats treated chronically with PPX

The effects of acute systemic PPX on VP neuronal firing rate were also assessed in non-treated control rats (n= 30) and rats that received 2mg/kg (±)PPX, ip, twice daily for 14 days followed by 2-4 drug-free days (n=27). To determine if the total number of neurons that responded to PPX, as well as the particular response category were altered by chronic PPX treatment, *Chi*-square analyses were conducted (see Table 8). No significant differences were obtained between the two groups for the number of responders *vs.* non-responders, nor a difference in the type of response displayed. We next analyzed the dose-response relationship between the non-treated control group and rats chronically treated with PPX (Fig. 25A). A two way repeated measures ANOVA revealed no effect of treatment group ($F_{(1,2176)}$=0.65, p=0.42), an effect of dose ($F_{(7,68403)}$=35, p<0.001), with no interaction ($F_{(7,3005)}$=1.5, p=0.16). This indicates there was a dose related response to (±)PPX, but chronic treatment with PPX does not affect this response. A *t*-test revealed no difference in the geometric E_{max} (Fig. 25B), but did find a significant difference between groups with respect to the geometric ED_{50} indicating that the potency of (-)PPX was increased in the PPX chronically treated group (Fig. 25C). *Chi*-square analyses revealed no difference between groups regarding the sensitivity for VP neurons to response at any of the doses tested (Fig. 26). However, there appears to be a trend for VP neurons in the chronic PPX group to respond to D3R-selective doses. This group had on average 50% more VP neurons that showed a response at the four lowest doses tested. On the contrary, there is a similar percent of neurons in each group responding at the three higher doses. This suggests D3Rs rather than D2Rs may be playing a role in the enhanced potency of PPX seen in the chronic PPX treatment group.

Effects of blocking D3Rs on PPX-induced changes in VP neuronal firing rate.

To ascertain if the effects of PPX on VP neuronal firing rates could be altered by the D3R-preferring antagonist, PG01037, a subset of intact rats (n=17) treated with the single acute dose of 300µg/kg (-)PPX were tested with 3mg/kg iv PG01037 (see Fig. 28 for the injection protocol and an example of treatment responses). Some rats also received 10 and 30mg/kg PG01037 in a cumulative fashion. Eight of the 14 rats tested with 30mg/kg unexpectedly died; thus, we only report effects of 3 and 10mg/kg PG01037. Of the 17 neurons tested with PPX, 10 neurons demonstrated a rate suppression. PG01037 at a dose of 3mg/kg antagonized (defined as attenuation of agonist-induced response by at least 20%) the agonist-induced effect in 8 of these neurons. A one way rmANOVA ($F_{(2,387)}$=13, $p<0.001$) determined that the firing rate was different across the three conditions (ie., during baseline, after the PPX injection, and after the 3mg/kg PG1037; Fig. 28). A *post hoc* Newman-Keuls revealed that PPX significantly decreased the firing rate compared to baseline levels and that 3mg/kg PG01037 significantly increased the firing rate that was altered by PPX. There was no significant difference between baseline firing rate and that following the 3mg/kg PG01037, indicating a reversal (defined as a re-establishment of baseline firing rate) of the PPX effects. Of these eight rats, seven then received a subsequent cumulative dose of 10mg/kg PG01037. A *t*-test comparing spikes per second after 3mg/kg (13.9 ± 3.8) and 10mg/kg (11.8 ± 2.8) PG01037 revealed no difference between these doses (p=0.42). Similar trends were seen (data not shown) in three neurons that demonstrated PPX-induced rate increases. It should be noted that of the 11 neurons that demonstrated antagonism with 3mg/kg PG01037, firing rate returned to baseline levels in 7 of these,

however, 6 of the neurons demonstrated firing rates that were different than baseline levels. Additionally there were 4 neurons that demonstrated no PPX-induced effects, but upon administration of 3mg/kg PG01037, one of the four neurons did show a change in firing rate. Overall, these data suggest that D3Rs activated by 300µg/kg (-)PPX can alter the neuronal firing of VP neurons as demonstrated in the directionality of PG01037-induced effects and also the ability to reverse firing rate back to baseline (i.e., pre-PPX) firing rate. However, there were clearly examples in which this was not the case, and thus *occasionally*, PG01037 appears to be influencing endogenous DA-induced effects. Therefore, caution should be taken when interpreting PG01037-induced effects.

Discussion

These experiments revealed the following: (1) Neither persistent 6-OHDA-induced lesions of the DLS or MFB, nor chronic PPX treatment altered basal activity of VP neurons as recorded in chloral hydrate-anesthetized rats. (2) Compared to controls, neither lesions of the DLS nor the MFB altered the potency or efficacy of PPX. (3) Chronic PPX increased the potency of PPX, as reflected in the capacity of low doses of the agonist to alter firing of VP neurons. (4) Effects of PPX up to 300µg/kg likely reflect a preponderance of D3R activation.

Lack of effect of dopamine denervation on basal activity of VP neurons

Some of the brain regions within the basal ganglia that are affected throughout the course of PD monosynaptically innervate the VP. These include the ascending dopaminergic system which directly regulates VP neuronal activity (Maslowski-Cobuzzi

and Napier, 1994; Mitrovic and Napier, 2002; Klitenick et al., 1992) and the STN which has a robust excitatory influence on VP neurons (Turner et al., 2001; Groenewegen and Berendse, 1990). The unilateral 6-OHDA-induced dopaminergic lesion of the MFB, a model of late stage PD, leads to near complete destruction of dopaminergic neurons (Heidenreich et al., 2004; Ungerstedt, 1968) and an increase in firing rate and oscillatory activity of the STN (Parr-Brownlie et al., 2007). In agreement with previous studies, we found that lesions of the ascending dopaminergic system did not alter the basal firing properties of VP neurons (Turner et al., 2002; Heidenreich et al., 2004). This suggests compensatory mechanisms are engaged during conditions of DA deafferentation that allow the VP to maintain a steady baseline firing profile.

The bilateral 6-OHDA-induced dopaminergic lesion of the DLS, a model of early stage PD, leads to a partial and discrete destruction of some nigral neurons (Blandini et al., 2007) and alterations in STN neuronal activity, including irregular pattern of firing, without a change in firing rate (Breit et al., 2007). Lack of change in basal spiking characteristics following this lesion may be due to insignificant changes in the circuitry that regulate VP neuronal spiking activity.

Lack of effect of chronic (±)PPX treatment on basal activity of VP neurons

Fourteen days of chronic PPX treatment leads to an increase expression of D3Rs in the NA (Maj et al., 2000; Tokunaga et al., 2012) and similar treatment paradigms with other D2/D3R preferring agonists such as 7-OH-DPAT and quinpirole enhance D3R expression and decrease expression of D2Rs in the VP (Stanwood et al., 2000b). Tonically released DA may allow these changes in D2/D3R to have a functional

consequence; however, our data suggest that any functional changes that may occur with the changes in expression of D2/D3Rs appear to be offset by additional compensatory mechanisms that allow the VP to maintain a steady baseline firing profile.

PPX induced both enhancements and suppressions in the firing rate of VP neurons

In control rats, systemic injections of PPX altered VP neuronal firing in approximately 50% of the neurons tested with similar proportions of rate enhancements and rate suppression measured. As PPX is given systemically in these studies, the final measured response of VP neuronal firing is likely be influenced by direct and indirect circuit related effects as well as pre and postsynaptically-mediated effects. Data collected by Piercey and colleagues with intact rats give us some indication of how the brain regions within the relevant circuitry under study are influenced by systemic PPX (Piercey et al., 1996). PPX inhibits activity of most midbrain neurons (Piercey et al., 1996), while PPX enhances neuronal activity in the caudate nucleus (Piercey et al., 1997). PPX is reported to predominantly inhibit neurons in the NA, but rate-enhancements as well as no effect on firing rate occurred (Piercey, 1998). There are, however, many unknowns regarding the influence of PPX on brain regions that express D2/D3Rs and that send direct projections to the VP. These include the AMG, STN and PFC, all of which send glutamate projects to the VP (Delgado-Martinez and Vives, 1993; Fuller et al., 1987; Groenewegen and Berendse, 1990; Turner et al., 2001; Mitrovic and Napier, 1998; Napier and Mitrovic, 1999; Bouthenet et al., 1991; Flores et al., 1999; Loiseau and Millan, 2009).

Data collected by Napier and colleagues also provides information about the consequences of exogenous and endogenous DA as well as the effects of D2R stimulation in the VP and the related circuitry. For example, microiontophoretically applied DA directly into the vicinity of VP neurons increases and decreases VP neuronal firing rate, with a larger proportion of neurons showing a rate suppression (Napier et al., 1991; Maslowski-Cobuzzi and Napier, 1994). Stimulation of the VTA or SN evokes a response from almost all VP neurons tested; rate enhancements are seen but there is double the number of rate suppressions (Maslowski-Cobuzzi and Napier, 1994). These firing rate alterations have been attributed to activation of both D1R and D2Rs located within the VP. Local application of D1R agonists decrease firing rates, while D2R agonists increase firing rates in VP neurons (Napier and Maslowski, 1994). When these agonists are delivered systemically the opposite effects are seen. That is, D1R agonists increase VP neuronal firing rate; whereas D2R agonists produce rate decreases (Maslowski and Napier, 1991; Napier, 1992; Heidenreich et al., 2004; Heidenreich et al., 1995). These results indicate that VP neuronal activity is influenced by direct and indirect circuit related effects. DA also acts a modulator within the VP. For example, electrical stimulation of the VTA attenuates glutamate-evoked VP responses induced by AMG stimulation (Maslowski-Cobuzzi and Napier, 1994). Also, co-iontophoresis of DA with either glutamate or GABA substantially alters both GABA and glutamate evoked responses in the VP, with attenuation of the evoked response occurring most often (Johnson and Napier, 1997). Based on the information that we do know, we can start to see the complexities of how systemic PPX, by acting on D2R and D3Rs, might alter neuronal firing in this circuitry and ultimately change the firing rate of VP neurons.

Given that the VP expresses both D2 and D3Rs, and it receives excitatory and inhibitory inputs from both midbrain dopaminergic neurons and other dopaminergic receptive brain region, it is not surprising that PPX induces a both firing rate enhancements and suppression VP neurons.

Given that PPX-induced responses in the current study were evaluated at doses that are reported to be acting at both D2/D3R, it might be expected that the PPX-induced responses would be similar to results found using D2-like receptor agonists. Our findings demonstrated that the absolute ED_{50} for PPX was between 150-200µg/kg; E_{max} was approximately 75% of baseline. This is in contrast to systemically administered quinpirole, a D2-like family receptor agonist, which has slight preference for the D3R over the D2R, (Pugsley et al., 1995; Chio et al., 1994; Sautel et al., 1995) although some studies suggest that it is nonselective between D2 and D3Rs (Mierau et al., 1995). Quinpirole has been shown to alter VP firing in 88% (45 of the 51) neurons tested; attenuation of the firing rate was predominately observed (Maslowski and Napier, 1991). The ED_{50} for quinpirole was 7.6µg/kg; E_{max} was approximately 60% of baseline (Maslowski and Napier, 1991). The larger abundance of D2R as compared to D3Rs within the VP and the related circuitry can explain the increased number of responders as well as the increased potency of quinpirole. The increased proportion of rate enhancements seen with PPX as compared to quinpirole may be the result of direct activation of D2/D3Rs in the VP as the Napier laboratory has demonstrated that microiontophoretic application of D2Rs agonists within the vicinity of individual recorded VP neurons results in firing rate enhancements (Napier and Maslowski, 1994). Compared to quinpirole, PPX may be slightly more selective for the D3R over the D2R

(Mierau et al., 1995; Collins et al., 2007), thus differences in effects of these two agonists on VP neuronal firing rate may be due to differential activation of D3R in the circuitry.

D3Rs are involved in PPX-induced changes in VP neuronal firing rate.

We used PG01037, one of the most D3R preferring antagonists available to date, to determine if D3Rs are involved in the PPX-induced changes in VP neuronal firing rate. This antagonist is 133x's more selective for the D3R over the D2R (Grundt et al., 2005), and has been shown *in vivo* to be D3R selective at 32 and 56mg/kg sc (Collins et al., 2005). Furthermore, when the dose range of 3-32mg/kg PG01037 was tested for effects on reward mediated behaviors, 10mg/kg ip and 32mg/kg sc were shown to attenuate motivation and reward seeking behaviors (Orio et al., 2010; Higley et al., 2011). Using this same dose range in our current studies, we found that the lowest dose of PG01037 (3mg/kg, iv) was able to antagonize and reverse the rate altering effects of 300µg/kg (-)PPX to baseline (i.e., pre-agonist treatment) levels. The 10mg/kg dose did not attenuate the firing rate any further. As mentioned in the results section, there were cases in which neurons demonstrated a response to PG01037 that would indicate the antagonist is acting on endogenously activated receptors (i.e., (i) a case in which 300ug/kg (-)PPX had no effect of firing rate, but PG01037 did, and (ii) when PG01037 induced a change in firing rate that reversed the effects seen following PPX but also further altered the firing rate so that is was different from baseline levels). Additionally we collected pilot data on the ability of PG01037 to antagonize the effects of a cumulative dose of 3mg/kg (-)PPX. These experiments were conducted in rats used in the current study, including those with 6-OHDA-induced lesions to DLS (n=12), 6-OHDA-induced lesions to MFB (n=3), and

rats chronically treated with PPX (n=15). Together with the 17 intact rats that received a single dose 300ug/kg (-)PPX, 56% of neurons tested with 3mg/kg PG01037 provide evidence that PG01037 is blocking the effects of a PPX, while 33% of the neurons tested provide evidence that PG01037 is acting on endogenously activated D2/D3Rs. The remaining 11% of neurons tested did not demonstrate an effect of 3mg/kg PG01037. These data indicate that in a majority of the neurons tested, the PG01037-induced effects are likely antagonizing PPX-induced effects. This is demonstrated in the directionality of PG01037-induced effects and also the ability to reverse firing rate back to baseline (i.e., pre-PPX) firing rate. However, there were clearly examples in which this was not the case, and occasionally, PG01037 appeared to be influencing endogenous DA-induced effects. Indeed, endogenous DA is released in the VP (Gong et al., 1998; Lavin and Grace, 1998; Sizemore et al., 2000; Maslowski-Cobuzzi and Napier, 1994; Napier et al., 1991), as well as other brain regions that may influence the firing rate outcome of VP neurons (i.e., PFC, AMG, NA; Bortolozzi et al., 2007; Volonte et al., 1995; Oshibuchi et al., 2009). Therefore, caution should be taken when interpreting PG01037-induced effects on PPX-induced changes in VP neuronal firing rate.

Unexpectantly, many rats died within minutes of an iv injection of 30mg/kg PG01037. There have been no reported rodent deaths with the use of higher doses of this antagonist when given ip or sc. Thus, it may be the sudden and excessive blockade of D3Rs in the periphery that underlies the mortality seen in our studies. DA plays many roles including regulation of blood pressure (Zhang et al., 2011). D3R activation can alter sympathetic tone by inducing both vasoconstriction and vasodilation (for review see (Zeng et al., 2007); therefore, PG01037 may have led to a sudden change in the

vasculature which might have caused heart failure (D3Rs in the kidneys function to increase urinary sodium excretion but this would not acutely effect blood pressure). Given the robust effects of the lowest dose of PG01037 to attenuate and in many cases reverse the effect of 300µg/kg (-)PPX, this may indicate these agonist effects are driven mainly by the D3Rs with little effect of PPX on D2R activation. In support of this conclusion, a pharmacologic magnetic resonance imaging study demonstrated that PG01037 at 2mg/kg iv increases regional cerebral blood volume in areas with the highest concentration of D3Rs (i.e., Islets of Calleja and the shell of the NA), while smaller changes in blood volume were seen in the caudate-putamen, a brain region that expresses less D3Rs but more D2Rs (Grundt et al., 2007).

Bilateral 6-OHDA-induced lesion to the DLS do not alter efficacy nor potency of PPX; however rats with a unilateral 6-OHDA-induced lesion to the MFB tended to demonstrate enhanced potency of PPX, with no effect on efficacy

We hypothesized that 6-OHDA-induced lesions to the MFB or to the DLS would alter the response of VP neurons to PPX and this would be reflected in a change in efficacy and potency of PPX. However, the data indicated a trend for enhanced potency of PPX in the 6-OHDA-MFB group. Given the changes in expression level of D2Rs and D3Rs reported to occur following a MFB lesion, this *potential* increased potency may be a consequence of the increased expression of D2Rs within the circuitry and unlikely a result of an increased number of D3Rs. This is supported by our findings that VP neurons in rats with 6-OHDA-induced lesions were significantly more sensitive (i.e., more likely to demonstrate a change in firing rate) to the larger more D2/D3R selective

doses of PPX compared to lower D3R selective doses. Despite these receptor adaptations, our findings suggest that such compensatory mechanisms do not alter the efficacy or potency of PPX to induce changes in VP neuronal firing.

Bilateral 6-OHDA-induced lesions to DLS produce partial and discrete destruction of some nigral neurons that occur in this lesion (Blandini et al., 2007). Such destruction is likely to lead to loss of D2/D3 autoreceptors. Our findings suggest that such compensatory mechanisms do not alter the efficacy or potency of PPX to induce changes in VP neuronal firing.

Chronic treatment with PPX enhanced the potency but did not affect the efficacy of PPX

We hypothesized chronic treatment with PPX would alter the response of VP neurons to PPX and this would be reflected in a change in efficacy and potency of PPX. Our data demonstrate that chronic treatment with PPX did not influence the efficacy of PPX as there was no change in E_{max} as compared to the non-treated control group. However, potency of PPX was enhanced.

A change in the number of receptors can influence the potency of a drug (Kramer et al., 2011). If spare receptors (i.e., a receptor reserve that is not required for generating a maximal response) are present, a change in receptor number may not alter the maximal effect, but could still affect the potency, which is what we saw in the current study. Alternatively, a change in potency can also be reflected in a change in efficiency of the receptor coupling to its associated G-protein or efficiency of the second messenger system. D2Rs, as well as D3Rs, are coupled to the $G_{i/o}$ protein and inhibit the formation

of the second messenger cyclic adenosine monophosphate (cAMP) by inhibiting adenylyl cyclase (Spano et al., 1978; Kebabian and Calne, 1979). The literature provides us evidence that D3R expression should increase in some regions of the brain following chronic treatment of a D3R-preferring agonist (Maj et al., 2000; Tokunaga et al., 2012; Stanwood et al., 2000b). Thus, if a receptor reserve exists for the population of neurons responsible for the effects of PPX on VP neuronal activity, the increase in D3R expression would not affect efficacy but could enhanced the potency of PPX. Additionally, it has been reported that mice chronically treated with PPX (1mg/kg sc) have superactivation of adenylyl cyclase in the NA (Maggio et al., 2009). Thus, the enhanced efficiency of the second messenger system in the NA maybe one mechanism by which the potency of PPX to induced a change in VP neuronal firing rates was enhanced in the current study.

Functional implications for enhanced potency of PPX in rats chronically treated with PPX

Although acute administration of PPX can alter aspects of decision making (Pizzagalli et al., 2008; Riba et al., 2008), it is likely that chronic treatment with the drug is necessary for ICDs to develop. Preclinical studies report different outcomes when comparing chronic *vs.* acute administration of PPX. For example, electrophysiology studies demonstrate differential changes in the mean firing rate as well as the burst firing activity of VTA dopaminergic neurons following a two day treatment with 1mg/kg PPX compared to a 14 day treatment (Chernoloz et al., 2009). Our lab previously reported a larger increase in risk-taking behavior following a 14 day chronic PPX treatment regimen

compared to a single injection (Rokosik and Napier, 2012). In the current study we used the same chronic PPX treatment and withdrawal time period as used in our prior work to determine if PPX-induced changes in VP neuronal firing are altered under such conditions. Our data reveal this treatment regimen enhanced the potency of PPX. Moreover our data suggest the enhanced potency may be reflected in an increased sensitivity of the circuitry to alter VP neuronal activity *via* activation of D3R in particular. Collectively, these data suggest a possible link between firing activity in VP and the enhanced risk-taking behavior observed in rats treated chronically with PPX.

One mechanism by which PPX may be inducing ICDs is by enhancing the motivational salience of cues associated with rewards. Animal studies show that 0.3 and 1.0 mg/kg PPX enhances salience of cues previously associated with cocaine (Collins et al., 2011). In addition, the Napier laboratory demonstrated that 2mg/kg (±)PPX induces a conditioned place preference indicating that the environmental cues associated with the rewarding effects of PPX gain salience and thus motivate the rat to seek them out (Riddle et al., 2012). In the current study, we demonstrated that these same doses of PPX alter VP neuronal firing rate. Studies show that firing rate of VP neurons is altered by rewards and their learned incentive cues (Tindell et al., 2004) and that VP neuronal firing rate encodes predictive and motivational information about rewards (Tindell et al., 2005). Moreover, in both human and rodent studies, the VP is activated by reward cues in (Tindell et al., 2009; Mahler and Aston-Jones, 2012; Tsurugizawa et al., 2012; Childress et al., 2008). A neuroimaging study detected a positive correlation between activation of the VP following appetizing food cues and ‘reward drive’ (Beaver et al., 2006). Activation of the VP was also detected during a human functional MRI study in which

there was increased motivational behavior in response to cues that predicted the potential gain of a large quantity of money (Pessiglione et al., 2007). Given these findings, we propose that the VP plays a role in mediating PPX-induced responses such that in the presence of PPX, the VP neuronal activity may show enhanced firing rates to reward-related cues, leading to greater salience attributed to the cues.

Another mechanism by which PPX may be inducing ICDs is by altering reinforcement learning (Pizzagalli et al., 2008; Riba et al., 2008; Cools et al., 2006; Abler et al., 2009), such that behavioral flexibility and response inhibition are compromised leading to perseveration on a no longer rewarding activity. The VP is in a position to influence reinforcement learning and behavioral flexibility via its influence on tonic DA release from the VTA to the NA (Floresco et al., 2003). Behavioral flexibility, or the ability to switch between tasks when rewarding outcomes are altered, is driven in part by projections from the PFC to the NA (Goto and Grace, 2005; Block et al., 2007). This cortical input is modulated by D2R activation by tonic DA release from the VTA, such that reduction in tonic D2R stimulation is essential to allow for task switching (Goto and Grace, 2005). Indeed it has been shown that VP activation facilitates PFC-evoked responses in the NA (Goto and Grace, 2005). Thus, it is possible that PPX-induced suppression of VP firing rate could disinhibit VTA neurons resulting in more spontaneously firing neurons and thus enhanced dopaminergic tone in the NA. This may explain why individuals with PPX-induced ICDS continue to engage in behaviors despite negative consequences.

Tonic levels of DA are also proposed to increase under conditions of uncertainty. For example, when the probability of receiving a reward is either 100% or 0%, there is no

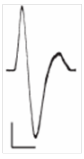
uncertainty. However, at 50% probability, uncertainty is at its highest. Schultz and colleagues have demonstrated a positive correlation between uncertainty and sustained increase in activation of midbrain neurons that occurs from the onset of a predictive cue to the expected time of reward (Fiorillo et al., 2003). The authors concluded that uncertainty itself contributes to the dopaminergic reward properties of gambling, which may explain why pathological gamblers continue in to engage in gambling activities despite losses. Assuming that VTA neurons contribute to this phenomenon, we suggest PPX-induced suppression of VP firing rate may allow for an even larger dopaminergic tone under conditions of uncertainty, thus propagating the motivation to gamble. Moreover, this effect will be enhanced in patients chronically medicated with PPX.

Summary and conclusions

The findings from intact rats in the current study established that (i) doses of PPX that increase reward motivated behavior engaged VP neurons (ii) at least at the dose of 300µg/kg, D3Rs mediated the ability of PPX to alter VP neuronal firing and iii) a chronic PPX treatment dosing protocol that increases risky behaviors also increased the potency of PPX to alter VP neuronal firing. We propose that one mechanism by which PPX may influence decision making in individuals with ICDs is through an increase in limbic D3Rs expression that, when activated by PPX, alters VP neuronal function. Activation of the VP leads to enhanced salience of reward-related cues and disruption in reinforcement learning. Although our findings indirectly support a role for the VP as well as D3Rs in the ability of PPX to enhance cue-induced reward seeking behaviors and well as gambling-like behaviors, they do provide support for this testable hypothesis. Thus, to

directly investigate the involvement of the VP in these behaviors, future site injection studies can ascertain if activation/inactivation of receptors in the VP is sufficient and/or necessary for these outcomes by using paradigms such as probability discounting and progressive ratio studies. To directly test the involvement of D3Rs, systemic injections and/or site injection studies can utilize the various D3R-preferring agonists and antagonists in these behavioral paradigms. Unfortunately, selective D3R compounds are still unavailable, making it difficult to tease apart the true involvement of D2R and D3Rs. Nevertheless, any advancement towards elucidating the neuropharmacology behind PPX-induced ICDS will be important for future development of medication that are beneficial for motor impairments in PD, but are devoid of the potential of inducing ICDs.

Table 4. Action potential characteristics from initially negative biphasic and initially positive triphasic baseline recordings in the VP; comparisons among the various control and treatment conditions.

		Non-treated control	Vehicle - DLS control	6-OHDA-DLS	6-OHDA - MFB	(±)PPX chronically treated	Combined control group
Biphasic 		N=31	N=17	N=19	N=23	N=19	N=48
	Amplitude (μV)[a]	454± 18	527 ± 38	461 ± 22	424 ± 23*	520 ± 32	459 ± 15^
	Duration (ms)	1.5 ± 0.03	1.5 ± 0.04	1.6 ± 0.04	1.6 ± 0.06	1.6 ± 0.05	1.5 ± 0.02^^
Triphasic 		N=17	N=7	N=10	N=14	N=8	N=23
	Amplitude (μV)[b]	804 ± 60	758 ± 121	945 ± 94	825 ± 112	1081 ± 84 #	766± 57
	Duration (ms)	1.3 ± 0.09	1.3 ± 0.14	1.5 ± 0.06	1.4 ± 0.1	1.4 ± 0.07	1.3 ± 0.08

Non-treated control column includes those rats that were tested in (-)PPX dose response evaluations and tested with a single dose of (-)PPX. Combined control group column includes all non-treated control rats and vehicle-DLS controls.

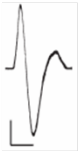
Green coded columns were compared (i.e. vehicle-DLS and 6-OHDA -DLS and 6-OHDA-MFB treatment groups) in a one way ANOVA. For biphasic amplitude, there was a treatment group effect, [A] $F_{(2)}=3.5$, $p=0.03$. *Post hoc* Newman Keuls * $p<0.05$, compared with vehicle-DLS. ANOVAs revealed no difference between treatment groups for biphasic duration ($F_{(2)}=0.7$, $p=0.5$), triphasic amplitude ($F_{(2)}=0.6$, $p=0.55$), nor triphasic duration ($F_{(2)}=0.3$, $p=0.73$).

Blue coded columns were compared (i.e., non-treated and (±)PPX chronically treated treatment groups) in a *t*-test. There was no difference between treatment groups for biphasic amplitude, ($t_{(44)}$=1.9, p=0.06), biphasic duration ($t_{(45)}$=0.89, p=0.37) nor triphasic duration ($t_{(23)}$=0.66, p=0.5). [b] There was a difference in triphasic amplitude [#]($t_{(22)}$=2.6 [#]p=0.01).

In the combined control groups, biphasic and triphasic action potential characteristics were compared using a *t*-test. There was a difference in amplitude ($t_{(64)}$=6.6 [^]p< 0.001) and duration ($t_{(66)}$=2.9 ^^p=0.004).

Action potential calibrations: horizontal bar = 1ms; vertical bar for biphasic action potential, 100µV; vertical bar for triphasic action potential, 125 µV.

Table 5. Spiking characteristics from initially negative biphasic and initially positive triphasic baseline recordings in the VP; comparisons among the various control and treatment conditions.

		Non-treated control	Vehicle - DLS control	6-OHDA- DLS	6-OHDA - MFB	(±)PPX chronically treated	Combined control group
Biphasic 	Rate (spikes/sec)	14 ± 1.6 (n=30)	13 ± 1.7 (n=17)	9.6 ± 1.0 (n=18)	10 ± 1.2 (n=23)	9.7 ± 1.0 (n=17)	13.6 ± 1.2 (n=47)
	Mean/Mode ISI	1.9 ± 0.2 (n=24)	1.67 ± 0.2 (n=13)	2.2 ± 0.4 (n=15)	1.7± 0.2 (n=6)	2.0 ± 0.3 (n=18)	1.8 ± 0.2 (n=37)
Triphasic 	Rate (spikes/sec)	15 ± 1.5 (n=17)	16 ± 3.8 (n=6)	11 ± 1.2 (n=10)	11 ± 1.7 (n=14)	12.8± 2.7 (n=8)	15.3 ± 1.5 (n=23)
	Mean/Mode ISI	1.5 ± 0.1 (n=9)	1.5 ± 0.3 (n=4)	1.3 ± .1 (n=8)	1..7± 0.1 (n=3)	1.6± 0.2 (n=8)	1.5 ± 0.1 (n=13)

Non-treated control column includes those rats that were tested in (-)PPX dose response evaluations and tested with a single dose of (-)PPX. Combined control group column includes all non-treated control rats and vehicle-DLS controls.

Green coded columns were compared (i.e. vehicle-DLS and 6-OHDA -DLS and 6-OHDA-MFB treatment groups) in a one way ANOVA. There was no difference between treatment groups for biphasic rate ($F_{(2)}$=1.7, p=0.19), biphasic ISI ($F_{(2)}$=1.25, p=0.07), triphasic rate ($F_{(2)}$=1.6, p=0.21), nor triphasic ISI ($F_{(2)}$=0.58, p=0.57).

Blue coded columns were compared (i.e., non-treated and (±)PPX chronically treated treatment groups) in a *t*-test. There was no difference between treatment groups for

biphasic rate ($t_{(48)}$=1.3, p=0.19), biphasic ISI ($t_{(40)}$=0.25, p=0.8) triphasic rate ($t_{(22)}$=0.44, p=0.66), nor triphasic ISI ($t_{(15)}$=0.38, p=0.7).

In the combined control groups, biphasic and triphasic spiking characteristics were compared using a *t*-test. There was no difference in rate ($t_{(68)}$=0.87, p< 0.38) nor ISI ($t_{(48)}$=1.2 , p=0.24).

Action potential calibrations: horizontal bar = 1ms; vertical bar for biphasic action potential, 100µV; vertical bar for triphasic action potential, 125 µV.

Table 6. Summary of neuronal responses to systemic injection of 300µg/kg (-)PPX given either as a cumulative divided dose or a single dose.

Dosing protocol	Response category	% of responders	Magnitude of response
Cumulative divided dose	Increase	27% (14/52)	+76.3 ± 14.7%
	Decrease	19% (10/52)	-52.5 ± 5.9%
	No change	53% (28/52)	
Single dose	Increase	27% (13/48)	+53.4 ± 10.3%
	Decrease	35% (17/48)	-49.9 ± 7.9%
	No change	38% (18/48)	

Chi-square analysis of the distribution of responses following the administration of acute (-)PPX either as a cumulative divided dose or as a single dose indicated that the number of cells that had an increase, decrease, or no response was not significantly different ($\chi^2_{(2)}$=3.87; p =0.14). Furthermore, a *t*-test revealed that the magnitude of response for VP neurons that demonstrated a rate increase or rate decrease was not significantly different between the two dosing protocols (Increase: $t_{(26)}$= 1.3, P = 0.21) (Decrease: $t_{(25)}$= 0.3, p = 0.79). Data for the magnitude of response are presented as the mean ± SEM.

Table 7. Summary of neuronal responses to systemic injection of 300µg/kg (-)PPX in vehicle-DLS, 6OHDA-DLS and 6OHDA-MFB treated rats.

Treatment condition	No. neurons sensitive to (-)PPX vs no. tested	Response category			
		Increase	Decrease	biphasic	No change
Vehicle-DLS	10/24	7	3	0	14
	(42%)	(29%)	(13%)	(0%)	(58%)
6OHDA-DLS	11/29	5	3	3	18
	(38%)	(17%)	(10%)	(10%)	(62%)
6OHDA-MFB	22/37	9	10	3	15
	(59%)	(24%)	(27%)	(8%)	(41%)

Using the drug administration protocol shown in Fig. 3, a drug-induced response was considered to have occurred if firing rate changed from baseline by at least 20% during two applications of the agonist with the inclusion of the 300µg/kg dose. Biphasic responses met the criteria for both an increase and decrease (and *vice versa*) in activity during the course of the dosing protocol. *Chi*-square analysis of the distribution of neurons sensitive to acute (-)PPX in the three treatment groups indicated no significant difference in the number of cells that fell in to these two categories ($\chi^2_{(2)}$=3.5; p =0.17). *Chi*-square analysis of the distribution of responses following the administration of acute (-)PPX in the three treatment groups indicated that the number of cells that had an increase, decrease, biphasic or no response was not significant ($\chi^{2(6)}$=7.8; p =0.25). Thus, treatment with 6-OHDA did not alter the neurons ability to respond to acute (-)PPX administration, nor the response distribution.

Table 8. Summary of neuronal responses to systemic injection of 300µg/kg (-)PPX in non-treated and chronic (±)PPX treated rats.

Treatment condition	**No. neurons sensitive to (-)PPX vs. no. tested**	**Response category**			
		Increase	Decrease	biphasic	No change
Non-treated	17/30	9	8	0	13
	(57%)	**(30%)**	**(27%)**	**(0%)**	**(43%)**
(±)PPX chronically treated	18/27	6	9	3	9
	(67%)	**(22%)**	**(33%)**	**(11%)**	**(33%)**

Refer to table 6 for explanation of categories. *Chi*-square analysis of the distribution of responses following the administration of acute (-)PPX in the two groups indicated that the number of cells that fell into these two categories were not statistically different ($\chi^2_{(1)}$=5.9; p =0.44). *Chi*-square analysis of the distribution of responses following the administration of acute (-)PPX in the two condition groups indicated that the number of cells that had an increase, decrease or biphasic or no response was not significant ($\chi^2_{(3)}$=4.24; p =0.24). Thus, chronic treatment with (±)PPX did not alter the neurons ability to respond to acute (-)PPX administration, nor did it alter the response distribution to acute (-)PPX administration.

A. B.

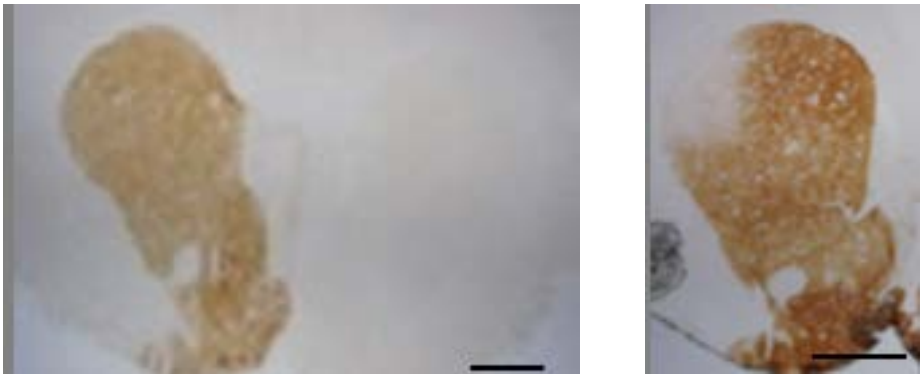


Figure 18. Validation of 6-OHDA-induced lesions. Photomicrographs showing representative immunohistochemistry staining for tyrosine hydroxylase (TH) in coronal sections of the striatum (~1.0mm anterior to bregma) following 6-OHDA or vehicle infusions. **(A)** A unilateral injection of 6-OHDA into the medial forebrain bundle reduced TH-like staining in the entire striatum of the hemisphere (right) compared to the vehicle treated side (left), twenty-five days following the injection surgery. For electrophysiological experiments, recording were only conducted on the lesion side of the brain. **(B)** Bilateral injections of 6-OHDA into the dorsolateral striatum reduced staining in a discrete area of the striatum twenty-one days following the injection surgery (shown is a unilateral section). The photomicrograph is representative of the lesion extent and location.

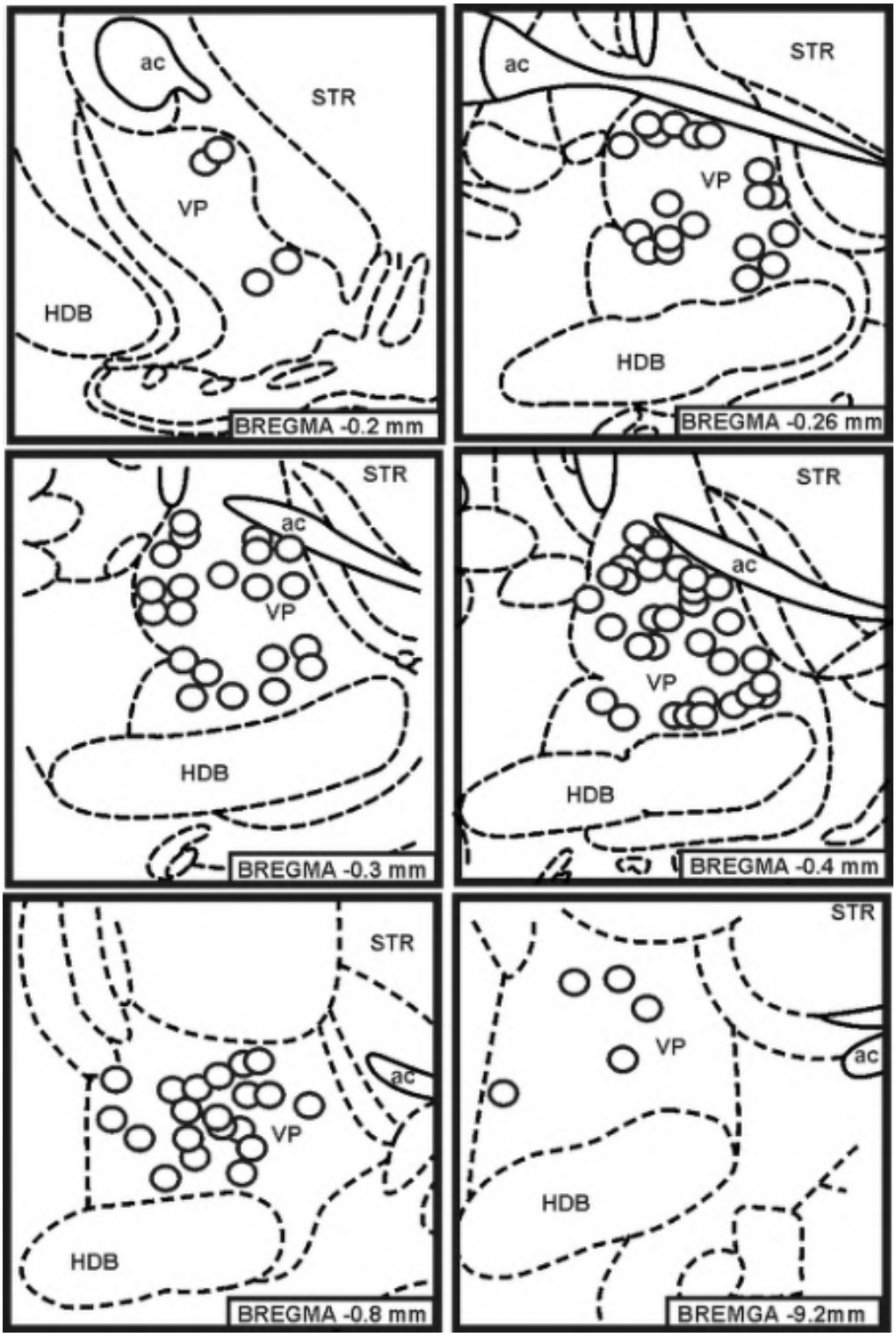

ac
STR
VP
HDB
BREGMA -0.2 mm
ac
STR
VP
HDB
BREGMA -0.26 mm
STR
ac
VP
HDB
BREGMA -0.3 mm
STR
ac
VP
HDB
BREGMA -0.4 mm
STR
ac
VP
HDB
BREGMA -0.8 mm
STR
VP
ac
HDB
BREMGA -9.2mm

Figure 19. Illustration of placement of recording sites in the VP. Recordings were made in both hemispheres, but they are collapsed into one hemisphere for illustration purposes. Recording cites (circles) are illustrated on six neuroanatomical plates modified from Paxinos and Watson (1998). ac, anterior commissure; HDB, horizontal limb of the diagonal band; STR, striatum; VP, ventral pallidum.

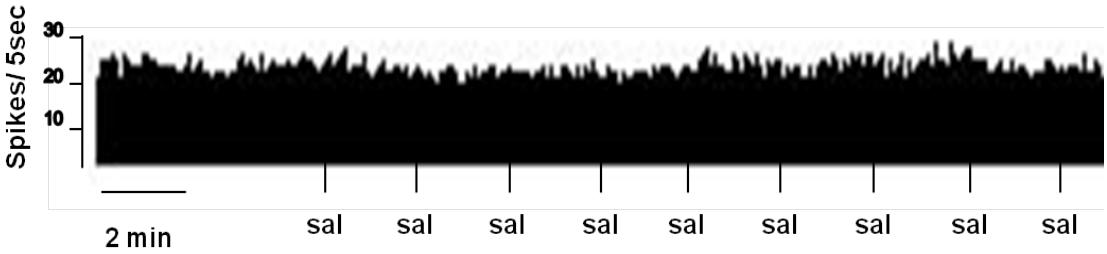


Figure 20. Cumulative rate histogram illustrating no change in VP neuronal firing rate (i.e., spikes per second) following multiple injections of saline vehicle. Following a five minute stable baseline recording, nine saline (sal) injections, given at a volume of 0.8µl each, were administered intravenously in approximately two minute intervals.

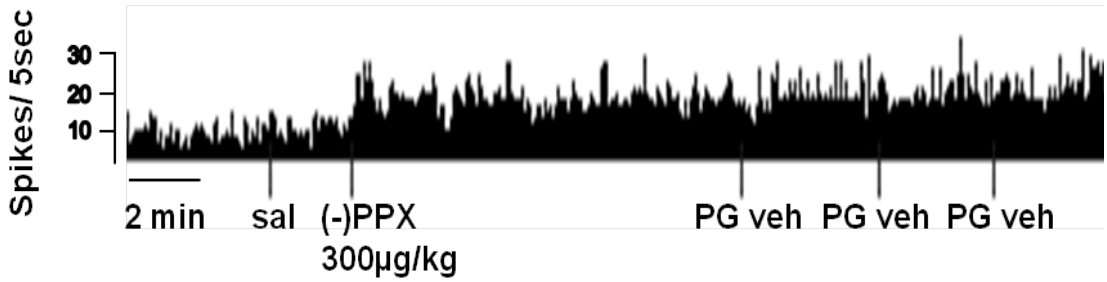


Figure 21. Cumulative rate histogram illustrating stability of a (-)PPX-induced change in VP neuronal firing rate (i.e., spikes per second) over time as well as lack of effect of multiple PG01037 vehicle (PG veh) injections on this agonist-induced response. Following a five minute stable baseline recording, saline (sal) was injected, two minutes later a single injection of 300µg/kg (-)PPX was administered and the firing rate was recorded for 15 minutes. This was followed by three intravenous injections of the PG01037 vehicle , which were administered in five minute intervals. All injection volumes were 0.8µl.

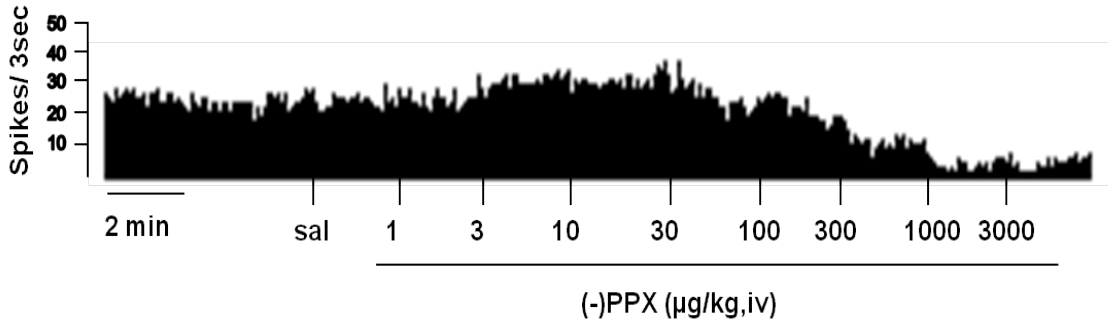
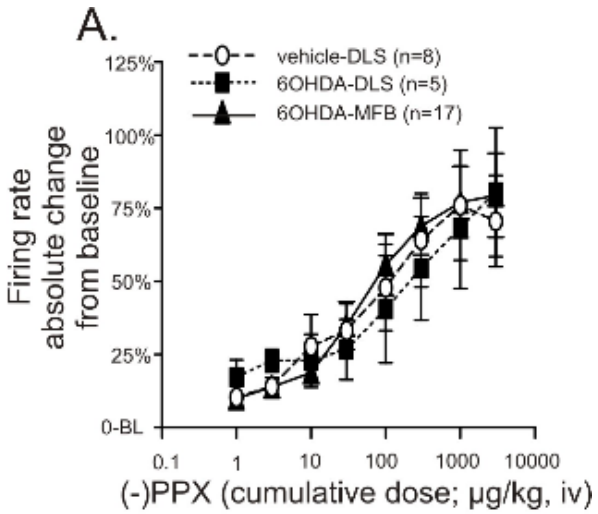
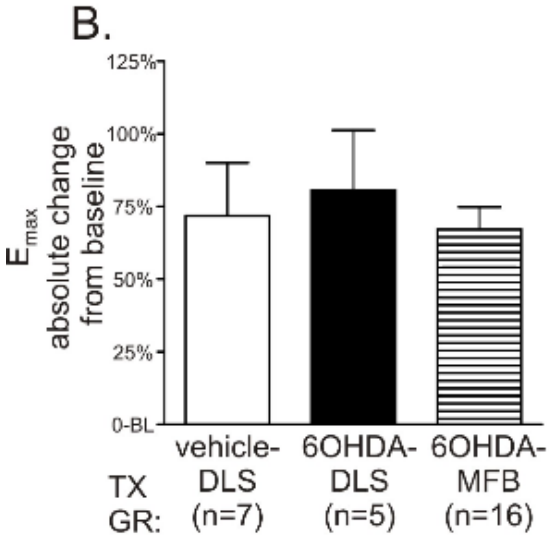


Figure 22. Cumulative rate histogram illustrating a (-)PPX-induced suppression of VP neuronal firing rate (i.e., spikes per second). Following a five minute stable baseline recording, saline (sal) was injected, two minutes later cumulative doses of (-)PPX were administered at two minute intervals.


A.
vehicle-DLS (n=8)
6OHDA-DLS (n=5)
6OHDA-MFB (n=17)
Firing rate
absolute change
from baseline
125%
100%
75%
50%
25%
0-BL
0.1
1
10
100
1000
10000
(-)PPX (cumulative dose; µg/kg, iv)


B.
E max
absolute change
from baseline
125%
100%
75%
50%
25%
0-BL
vehicle-
DLS
(n=7)
6OHDA-
DLS
(n=5)
6OHDA-
MFB
(n=16)
TX
GR:

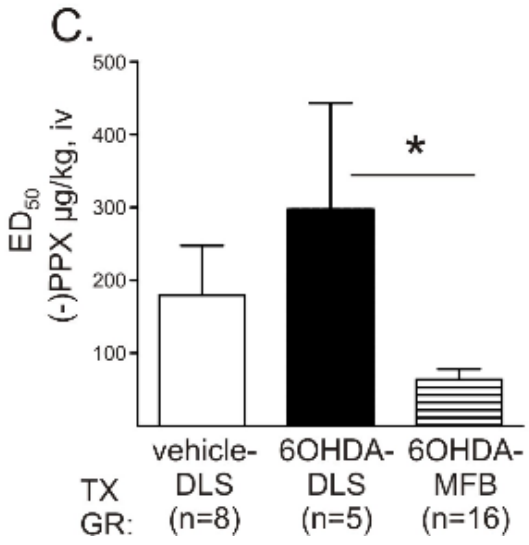

C.
ED50
(-)PPX µg/kg, iv
500
400
300
200
100
*
vehicle-
DLS
(n=8)
6OHDA-
DLS
(n=5)
6OHDA-
MFB
(n=16)
TX
GR:

Figure 23. Effects of 6-OHDA-induced lesions on the (-)PPX-induced changes in firing rate of VP neurons. Data were transformed into percent of the absolute change from baseline firing. Neurons were included in this analysis only if the goodness-of-fit (r^2) for a third order polynomial of their drug-response relationship was equal to or greater than 0.7. **(A)** Population average dose-response curves for VP neurons that demonstrated a change in firing rate in response to multiple iv injections of (-)PPX. Neurons were included in this analysis only if the goodness-of-fit (r^2) for a third order polynomial of their drug-response relationship was equal to or greater than 0.7. E_{max} and ED_{50} were determined from the individual dose-response curves and then averaged (see Materials and Methods) and differences between treatment groups (TX GR) were analyzed with a one way ANOVA. **(B)** E_{max} was not significantly different between neurons in the three treatment groups ($F_{(27)}$=0.23, p= 0.79). **(C)** For ED_{50} there was a significant effect of treatment group ($F_{(27)}$=4.0, p= 0.03). Newman-Keuls *post hoc* analysis revealed no difference between the vehicle-DLS group and neither the 6-OHDA-DLS nor the 6-OHDA-MFB group; however there was a difference between the two 6-OHDA treated groups ($p<0.05$).

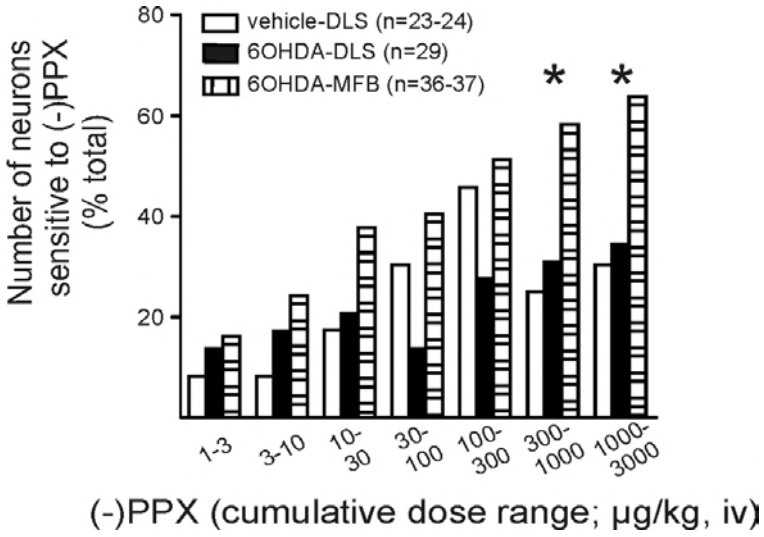
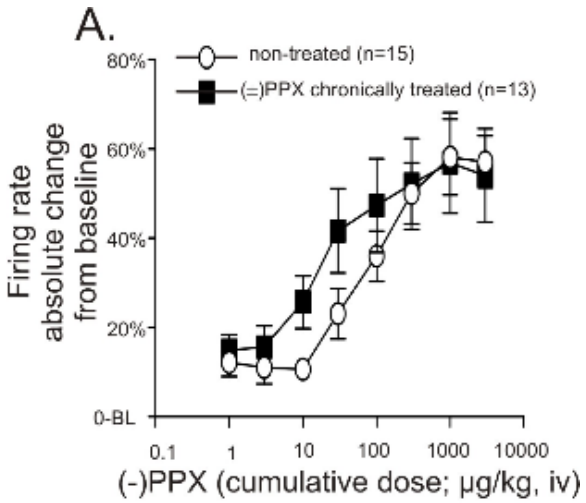
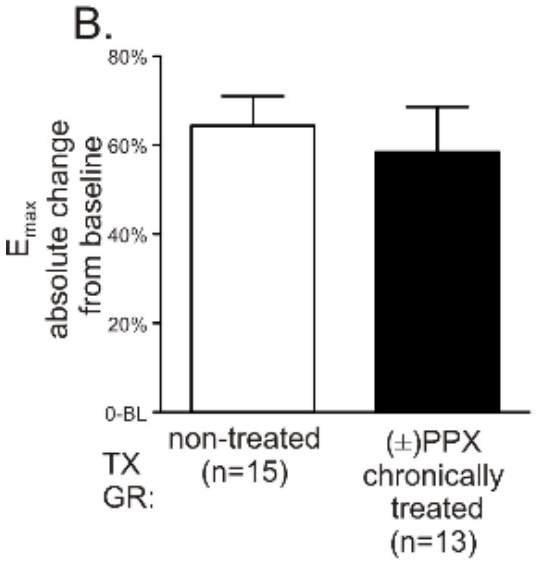


Figure 24. Sensitivity of VP neuronal firing to (-)PPX in vehicle-DLS, 6OHDA-DLS and 6OHDA-MFB treated rats. At each dose of (-)PPX, the ability for the agonist to change the firing rate of neurons was evaluated. A change in firing rate was considered to have occurred if there was a 20% change from baseline at that particular dose as well as at the following dose. *Chi*-square analysis of the distribution of percent of neurons that showed a response for each dose revealed a significant difference between groups in the two highest doses only (* $p \leq 0.02$). This indicates that a significantly higher percent of VP neurons in rats with a MFB 6-OHDA- treatment were sensitive to the highest doses of (-)PPX tested.


A.
non-treated (n=15)
(±)PPX chronically treated (n=13)
Firing rate absolute change from baseline
80%
60%
40%
20%
0-BL
0.1
1
10
100
1000
10000
(-)PPX (cumulative dose; µg/kg, iv)


B.
E_{max} absolute change from baseline
80%
60%
40%
20%
0-BL
TX GR:
non-treated (n=15)
(±)PPX chronically treated (n=13)

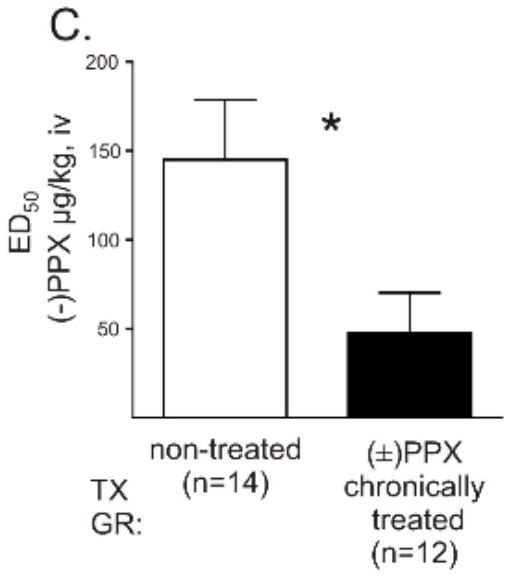

C.
ED_{50} (-)PPX µg/kg, iv
200
150
100
50
*
TX GR:
non-treated (n=14)
(±)PPX chronically treated (n=12)

Figure 25. Effects of chronic (±)PPX treatment on the (-)PPX-induced changes in firing rate of VP neurons. Data were transformed as a percent of the absolute change from baseline firing. Neurons were included in this analysis only if the goodness-of-fit (r^2) for a third order polynomial of their drug-response relationship was equal to or greater than 0.7. **(A)** Population average dose-response curves for VP neurons that demonstrated a change in firing rate in response to multiple iv injections of (-)PPX. Neurons were included in this analysis only if the goodness-of-fit (r^2) for a third order polynomial of their drug-response relationship was equal to or greater than 0.7. E_{max} and ED_{50} were determined from the individual dose-response curves and then averaged (see Materials and Methods) and differences between treatment groups (TX GR) were analyzed with a one way ANOVA. **(B)** E_{max} was not significantly different between the two groups ($t_{(26)}$ =0.49, p= 0.62). **(C)** For ED_{50} there was a significant difference between the two groups ($t_{(24)}$= 2.32, * p=0.03).

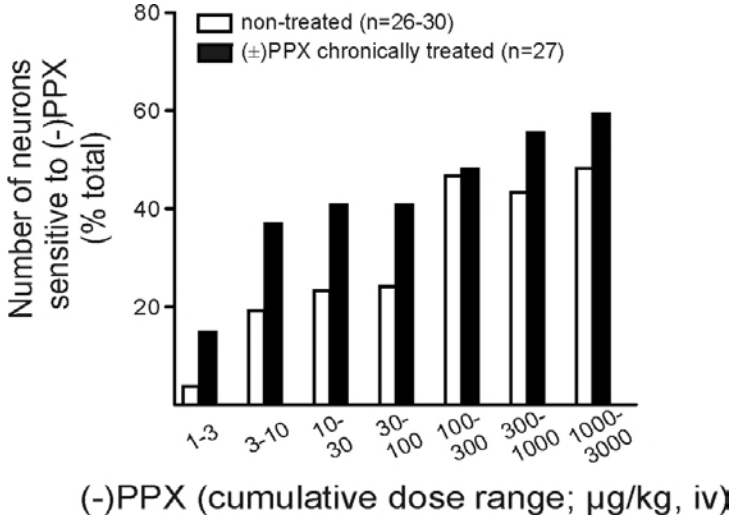


Figure 26. Sensitivity of VP neuronal firing to acute (-)PPX (iv) in non-treated and (±)PPX (ip) chronically treated rats. At each dose of (-)PPX, the ability for the agonist to change the firing rate of neurons was evaluated. A change in firing rate was considered to have occurred if there was a 20% change from baseline at that particular dose as well as the following dose. *Chi*-square analysis of the distribution of percent of neurons that showed a response for each dose revealed no significant difference for any dose ($p>0.2$ for all doses). However, there was a trend for neurons in the (±)PPX chronically treated group to be more sensitive to lower doses of (-)PPX tested.

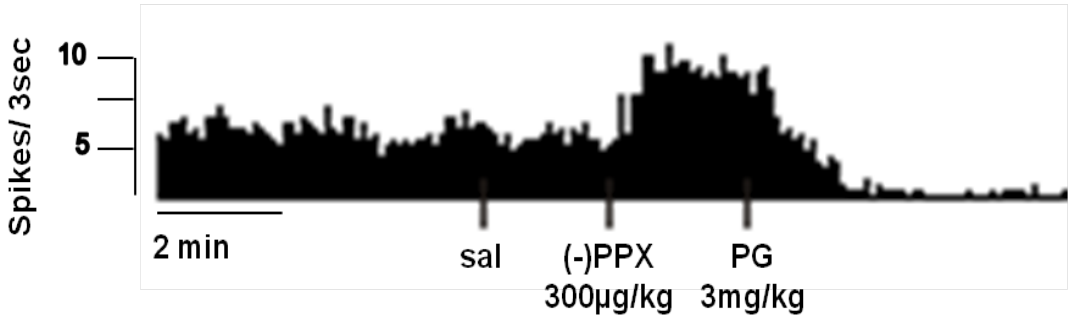


Figure 27. Cumulative rate histogram illustrating a (-)PPX-induced enhancement of VP neuronal firing rate (i.e., spikes per second) followed by antagonism of this response by PG01037. Following a five minute stable baseline recording, saline (sal) was injected, two minutes later a single injection of 300µg/kg (-)PPX was administered. This was followed two minutes later with an injection of 3mg/kg PG01037, firing rate was recorded for five minutes.

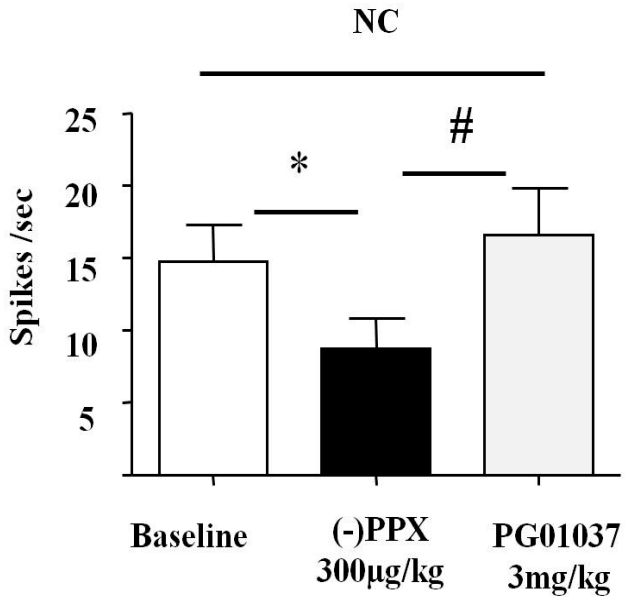


Figure 28. PG01037 reversed the (-)PPX-induced suppression of VP neuronal firing. For this group of neurons (n=10), the firing rate (spikes per second) was determined during baseline (BL) conditions, following administration 300µg/kg (-)PPX and subsequently following 3mg/kg PG01037. Compared to BL, 300µg/kg (-)PPX decreased the firing rate of VP neurons. This response was reversed by 3mg/kg PG01037. Data were analyzed with a one-way rmANOVA followed by a *post hoc* Newman–Keuls, $^{*}p < 0.05$ compared to Bl, $^{\#}p < 0.05$ compared to (-)PPX. Shown are the mean + SEM.

CHAPTER VI

GENERAL DISCUSSION: THE INFLUENCE OF PRAMIPEXOLE ON VENTRAL PALLIDAL ACTIVITY AND IMPULSIVE BEHAVIOR

ICDs induced by PPX are a serious side effect in treated PD patients (Weintraub et al., 2010). Animal models to study these effects will provide an invaluable means to study the underlying mechanisms driving PPX-induced ICDs and to use for medication development. Towards that end, the purpose of the first part of this dissertation project was to develop an animal model to study the effects of acute and chronic PPX on probability discounting, which is a measure of risk-taking, one aspect of ICDs. To determine if a PD-like brain state alters PPX-induced outcomes, we tested rats with dorsolateral striatal (DLS) lesions of dopaminergic terminals. Our studies revealed that acutely administered (±)PPX, in a moderately high dose of 2mg/kg, increases risk-taking. In both PD-like rats and controls, chronic treatment further augments this outcome. Thus, these studies revealed that a moderately high dose of PPX was able to induce probability discounting in PD-like rats and controls (Rokosik and Napier, 2012).

ICDs, such as gambling, are reward-based behaviors. Individuals with ICDs have a heightened motivation to engage in these behaviors. One mechanism by which PPX may alter the motivational state of an individual is by enhancing the salience of cues associated with the reward. Woods and colleagues reported that PPX increases the

reinforcing effects of cocaine-predicting cues, suggesting that PPX enhances the positive salience of the cues (Collins et al., 2011). Our laboratory revealed that PPX supports reward-mediated associative learning by inducing a conditioned place preference (Riddle et al., 2012). These two studies support the idea that PPX influences associative learning processes, including those that are used to atribute reward-related significance to previously neutral cues.

We are interested in the neurocircuitry underlying the ability of PPX to influence motivation and associative learning. Associative learning, as measured in place preference paradigms is regulated by several brain regions, including the VP (Dallimore et al., 2006; Mickiewicz et al., 2009; Gong et al., 1996). We contend that the VP may play a role in mediating aspects of PPX-induced ICDs. The following converging evidence from recent animal studies supports this: (**i**) Rewards and their predictive cues are represented in a firing rate code by neurons within the VP (Tindell et al., 2004). (**ii**) VP neural activity integrates predictive, incentive and reward value information (Tindell et al., 2005). (**iii**) VP neuronal activity encodes expected reward values (Tachibana and Hikosaka, 2012). (**iv**) VP neuronal activity is altered by both systemic (Maslowski and Napier, 1991; Napier, 1992; Heidenreich et al., 2004) and local application of DA and D2/D3R agonists (Napier and Maslowski, 1994; Napier et al., 1991; Maslowski-Cobuzzi and Napier, 1994). (**v**) Intra-VP injection of DA, or D1/D2R agonists alter behaviors in rats (Napier and Chrobak, 1992; Gong et al., 1996). Collectively these studies suggest that PPX may influence the activity of the VP and this may contribute to the PPX-induced alterations in the salience of reward-related cues.

People treated acutely or chronically with PPX demonstrate alterations in variety of impulsive behaviors (Pizzagalli et al., 2008; Riba et al., 2008; Housden et al., 2010; Voon et al., 2011a). Therefore, we investigated the ability of acutely administered and chronically administered (i.e., two weeks) PPX to alter VP neuronal firing rate using doses known to alter impulsive and other reward-related behaviors in rodents. As PPX is a D3R-preferring agonist and D3Rs within the limbic system drive reward-seeking behaviors, we also investigated the contribution of D3Rs in these PPX-induced alterations of VP neuronal firing rate. The data from these studies indicated that acute administration of PPX altered the firing rate of VP neurons. A D3R preferring antagonist was able to antagonize most of the VP neuronal firing rate alterations induced with 300µg/kg (-)PPX, which suggest that D3Rs activated by PPX is sufficient to alter the neuronal firing of VP neurons. In rats chronically treated with PPX, the potency of PPX to induce changes in VP neuronal firing rates was enhanced. Other laboratories have demonstrated that rats treated for 14 days with PPX (either using 0.3 or 1mg/kg, administered once daily) show increase expression of D3Rs in the NA (Maj et al., 2000; Tokunaga et al., 2012). We suspect that the enhanced expression of D3Rs in the NA and possibly other regions that express moderate to high levels of D3R (including the VP) may be an important mechanism underlying the enhanced potency seen in rats chronically treated with PPX. Chronic DA deafferentation can also alter expression of D2 and D3Rs. Drug naïve PD patients and rats with a 6-OHDA-induced lesion to the MFB show a decrease in D3R expression and an increase in D2R expression in the ventral striatum (Boileau et al., 2009; Bordet et al., 1997; Stanwood et al., 2000a; Levesque et al., 1995). The VP is altered in after 6-OHDA-induce DA deafferentation to the

ascending dopaminergic projections (Turner et al., 2002). In the studies conducted in this dissertation, we found near complete lesions of the ascending dopaminergic projections nor did discrete lesions of the DLS alter PPX-induced effects on VP neuronal firing rate. Thus, it does not appear that the neuroadaptations that occur following a 6-OHDA-induced late stage nor a 6-OHDA-induced early stage model of PD affects the ability of PPX to alter VP neuronal firing rate.

In summary, our behavioral studies indicate acutely administered PPX increases risk-taking behavior and chronic treatment further augments this outcome. A PD-like brain state does not appear to alter the ability of PPX to alter risk-taking behavior. In a step towards elucidating the neurocircuitry involved in PPX-induced risk-taking, we demonstrated the ability of PPX to alter firing activity of neurons in the VP, a brain region that codes for motivational aspects of rewards. A PD-like brain state did not affect these PPX-induced effects. We found the same chronic PPX treatment that enhanced risk-taking also enhanced the potency of PPX to alter VP neuronal firing rate. Given these findings, we propose that the VP may play a role in PPX-induced impulsivity.

Potential mechanisms underlying PPX-induced increases in risk-taking as measured in a probability discounting task

In this dissertation, we demonstrate that PPX can enhance risk-taking as measured in a probability discounting task (Rokosik and Napier, 2012). I propose that these effects can be due to the ability of PPX to alter at least two types of reinforcement learning that occur in this task. The first type of learning involves the generation of reward predictions errors. The second type of learning involves reversal learning.

As described in the Introduction, generation of reward prediction errors is a process by which an individual learns about the expectations of rewards. Positive unpredictable outcomes can generate seeking behavior, while negative outcomes, such as omission of rewards, can generate inhibition of behavior. Midbrain DA neurons can fire in different patterns to generate teaching signals (i.e. reward prediction errors) about positive and negative outcomes (Montague et al., 1996; Schultz et al., 1997; Pagnoni et al., 2002; Pessiglione et al., 2006; Cohen et al., 2012; Enomoto et al., 2011). For example, some of these neurons will fire in a phasic pattern when an unpredicted reward is encountered (i.e., a positive reward prediction error; Hollerman et al., 1998; Waelti et al., 2001). On the other hand, some of these neurons will show a transient depression in baseline rate of activity (thought to be a pause in the spontaneous 'tonic' firing (Grace and Bunney 1984)), when an expected reward is not delivered (Schultz, 2002; Bayer et al., 2007; Tobler et al., 2003). The different firing patterns and subsequent patterns of DA release differentially activate D1-like and D2-life family of receptors. Tonic and phasic release of DA is thought to act on D2R and D1Rs, respectively (Grace, 1991).

Learning about the occurrence of rewards and thus generating reward prediction errors is fundamental to a probability discounting task. For example, during this task phasic activation of midbrain DA neurons are predicted to signal changes in probability, such that anticipation of stimuli with larger reward probability elicits larger DA bursts, while anticipation for stimuli with smaller reward probability elicits smaller DA bursts (Fiorillo et al., 2003). Moreover, it is predicted that sustained activation of midbrain DA neurons, thought to provide a tonic DA release, will occur with uncertainty (Fiorillo et al., 2003). During the probability discounting, we randomized changes in the probability

of delivery of the large reinforce, thus learning is ongoing as conditions are constantly changing due to the changes in probability and certainty.

Phasic and tonic patterns of DA release and subsequent receptor activation are thought to be able to alter reward-mediated behavior by at least two ways. First, Frank and colleagues (2008) propose a model that incorporates the indirect and direct pathways of the basal ganglia (see Fig. 1). They propose phasic SNpc DA release following unexpected rewards activates the direct pathway (i.e., 'Go' pathway) by stimulating D1Rs, and thus leading to behavioral activation. Omission of rewards reduces the tonic DA levels that reduce stimulation of D2Rs in the indirect pathway (i.e., 'No-Go' pathway), and thus leading to suppression of behavior.

A second way in which patterns of DA release from VTA neurons can alter behavioral activation is *via* activation of D1 and D2R in the NA. The NA is involved in behavioral activation to rewards and reward-related cues (for review see (Nicola, 2007). This type of behavioral activation ties into the second type of learning that PPX may be affecting in the probability discounting task, which is reversal learning. Reversal learning incorporates behavioral flexibility so that an individual can inhibit a behavior that is no longer reinforcing and switch to a different behavior. The NA receives converging inputs from limbic and cortical regions (Groenewegen et al., 1999; French and Totterdell, 2002; French and Totterdell 2003; O'Donnell and Grace, 1995). These inputs will influence either behavioral activation or behavioral inhibition, respectively. Limbic regions such as the hippocampus and amygdala carry information regarding contextual and emotional information about the rewards (Everitt et al., 1999; Tabuchi et al., 2000), whereas the PFC monitors of choice information to adjust behavior in response

to a decision (Rogers et al., 2004; Marsh et al., 2007). By integrating the information from limbic and cortical inputs, the NA can relay this information to the VP and then to the motor outputs (see Fig. 3).

DA input from the VTA can modulate the type of information processed by the NA. For example, D2R stimulation in the NA attenuates the input from the mPFC to NA (O'Donnell and Grace, 1994; West and Grace 2002; Goto and Grace, 2005). Moreover, it was demonstrated that D2R stimulation is driven specifically by tonic DA release from the VTA. Conversely, phasic DA release and D1R stimulation increases the input from the hippocampus (Goto and Grace, 2005). Dopaminergic modulation NA transmission has behavioral consequences. Using a reversal learning task, Grace and colleagues (2005) found that D1R-mediated hippocampal input to the NA was critical for acquiring the task but D2R-mediated PFC input to the NA was critical for actually reversing a learned behavior. The authors found an increase or decrease in tonic D2R activation produced a suppression or facilitation of PFC input, respectively. In other words, a decrease in tonic D2R activation, which allowed PFC input to be processed by the NA was necessary for behavioral inhibition and subsequent reversal learning by the animals. Collectively these studies highlight the integrative nature of accumbal influences in behavior selection and how DA can modulate the information processed in the NA, and thus influence behavior.

Our behavioral results show that during baseline probability discounting (i.e., pre-PPX) rats prefer a small certain reward as the probability of delivery of the large reinforcer decreases. It is likely that the negative outcomes (i.e., selection of the risky lever with no rewarding outcome) during the low probabilities generate a negative reward

prediction error. Therefore, a pause in tonic DA release from the SN would occur, reducing stimulation of D2Rs and generating a 'No-Go'signal. Additionally, a pause in tonic DA release from the VTA would lead to a decrease in tonic DA stimulation of D2Rs in the NA, which would facilitate the PFC input to the NA and guide behavior to disengage from the no-longer rewarding situation.

Following administration of PPX, rats demonstrate an increase in risk-taking, such that even when the probability of delivery for the large reinforcer is low, rats continue to prefer that lever (Rokosik and Napier, 2012). I suspect that PPX may have blocked the 'DA dip' learning signal so that the probability of obtaining the reward was no longer a factor and/or the ability to disengage in risky lever selection was compromised due to suppression of PFC input to the NA. Frank and colleagues have hypothesized that D2R stimulation would impair the teaching signal propagated by DA dips (Frank and O'reilly, 2006; Frank and Hutchison, 2009). Indeed they demonstrated that carriers of the C957T polymorphism within the D2R gene, which is associated with increased striatal D2R density displayed deficits in learning from negative outcomes (Frank et al., 2007a). Work by Floresco and colleagues has also demonstrate that increases in D2R activity in the NA via microinjections of quinpirole causes a general impairment in reversal learning, which they related to attenuation of PFC input to the NA (Haluk and Floresco, 2009).

Several humans studies suggest that PPX can disrupt learning from negative outcomes when tested in probabilistic choice tests (i.e., when a reward is expected but not delivered) (Pizzagalli et al., 2008; Riba et al., 2008; Abler et al., 2009; Cools et al., 2006; Bodi et al., 2009). Evidence for PPX-induced alterations in reward learning was

demonstrated in a study by van Eimeren and colleagues who assessed the OFC (van Eimeren et al., 2009), a region of the PFC that is particularly necessary for learning from unexpected outcomes (Takahashi et al., 2009). They found that dopaminergic stimulation with PPX increased OFC activity during negative reward predictions, thus preventing decreases in DA transmission that occur with negative feedback. The authors suggest that pathological gambling may be associated with an impaired capacity of the OFC to guide behavior when faced with negative consequences (van Eimeren et al., 2009).

Collectively, our data, along with reports from human and animal studies strongly suggest that PPX is interfering with the ability to learn from negative outcomes. This likely reflects the ability of PPX, by activating D2-like family of receptors, to impair reward prediction errors as well as behavioral flexibility.

Acute effects of PPX: setting the stage for development of ICDs

Experimental tasks, such as the probability discounting paradigm, is useful for determining the effects of PPX on reward-related learning in both humans and laboratory animals. However, ICDs are complex and while the development of these disorders may start with aberrant reward learning, other factors must also be propagating these behavioral addictions. For example, a study by Abler and colleagues (2009) found that patients treated chronically with DA agonists, including PPX, demonstrated increases in fMRI signaling in the ventral striatum during trials in which expected rewards were omitted. However, they noted that none of the patients developed an ICD (Abler et al., 2009). This outcome underscores the fact that reductions in discounting or risky

behaviors is not equivalent to developing an ICD *per se* but likely represents a particular aspect of these complicated disorders.

Initial administration of PPX may be setting the stage for reward seeking behaviors. For example, PPX activates autoreceptors of the midbrain neurons. In rats, acute or short term treatment of PPX (2-day continuous release of 1mg/kg/day) decreases spontaneous firing of midbrain neurons (Chernoloz et al., 2009; Piercey et al., 1996). This could explain a report by Riba and colleagues (2008), in which they reported hypoactivation of the striatum in healthy individuals given an acute dose of PPX. These findings are line with the "reward deficiency syndrome" suggested by Blum and colleagues (1995) to explain addictions. It is thought that individuals with such a syndrome have a below normal level of dopaminergic stimulation and engage in activities that can boost DA in the mesolimbic system (Blum et al., 1995). While drug addicts take their drug of choice to boost DA levels, pathological gamblers engage in risky behaviors with unpredictable wins that can lead to brief increases in DA due to increase DA burst activity (Hollerman et al., 1998;Waelti et al., 2001) or engage in activities with uncertainty which can briefly elevate tonic levels of DA (Fiorillo et al., 2003). In the case of DA agonist-induced ICDs, one possible explanation for the *initial* appearance of ICDs may be due to the agonist inducing a reward deficiency syndrome in so much as initial treatment with PPX induces hypoactivation of reward circuits.

A role for the VP in PPX-induced alterations in reinforcement learning

I contend that the VP plays a role in mediating aspects of PPX-induced alterations in reward based behaviors, in part by affecting reinforcement learning. For example,

GABAergic transmission from the VP directly regulates population activity in the VTA, such that the VP can hold the dopaminergic neurons in an inactive state (Floresco et al., 2003). This places the VP in position to influence reward prediction errors. Our studies demonstrated that PPX, given acutely, and in a range of behaviorally relevant doses, can alter the firing rate of VP neurons; PPX-induced rate increases and decreases were observed. The ability of PPX to alter VP firing rate may be a mechanism by which PPX interferes with reward prediction errors. For example, the ability of PPX to enhance firing rates of VP neurons may inhibit VTA neurons from being able to respond to unpredictable rewards. Indeed phasic DA firing occurs only in those neurons that are spontaneously active (Floresco et al., 2003). On the other hand, PPX-induced suppression of VP neurons may disinhibit more VTA neurons leading to an increase in striatal DA efflux, which represents the tonic level of DA. This outcome has been shown to occur following inactivation of the VP (Floresco et al., 2003). In this situation, enhanced tonic DA levels may interfere with the ability of the brain to process reward omissions.

Tonic levels of DA are also proposed to increase under conditions of uncertainty. Schultz and colleagues have demonstrated a positive correlation between uncertainty and sustained increase in activation of midbrain neurons that occurs from the onset of a predictive cue to the expected time of reward (Fiorillo et al., 2003). The authors concluded that uncertainty itself contributes to the dopaminergic reward properties of gambling, which may explain why pathological gamblers continue in to engage in gambling activities despite losses. Assuming that VTA neurons contribute to this phenomenon, we suggest that PPX-induced suppression of VP firing rate may allow for

an even larger dopaminergic tone under conditions of uncertainty, thus propagating the motivation to gamble.

The VP is in a position to also influence behavioral flexibility and reversal learning *via* its influence on tonic DA release from the VTA to the NA (Floresco et al., 2003). For example, it has been shown that VP activation facilitates PFC-evoked responses in the NA (Goto and Grace, 2005). Thus, it is possible that PPX-induced suppression of VP firing rate could disinhibit VTA neurons resulting in more spontaneously firing neurons and thus enhanced dopaminergic tone in the NA. The combination of enhanced dopaminergic tone which would preferentially be activating the D2Rs, as well as the presence of PPX activating D2Rswould suppress PFC-evoked responses in the NA, thus compromising self-control.

The VP, *via* its connections to motor output structures such as the PPN (Tsai et al., 1989) and SNpr (Maurice et al., 1997) can influence locomotor activity (Napier and Chrobak, 1992) (see Fig 4). The VP receives a dense GABAergic input from the NA (Nauta et al., 1978) thus creating a pathway for limbic-motor integration (Mogenson et al., 1980). Given this point of integration, modification of reward-mediated locomotion is likely to occur, in part, *via* this VP-NA pathway. Dopaminergic inputs, particularly from the VTA, can influence the NA-VP pathway. Both the NA (Phillipson and Griffiths, 1985) and the VP (Klitenick et al., 1992) receive direct dopaminergic input from the VTA. Given the presence of D2-like receptors in all three brain regions (i.e.,VTA, NA and VP), it is possible that PPX has the potential to alter this circuitry and thus alter reward-mediated locomotion particularly when detection of reward prediction errors occurs.

Predicting the influence of PPX on the VTA-NA-VP pathway and subsequent changes in locomotor activity, all in the context of reward-motivated behavior, requires an understanding of how DA and dopaminergic agonists acting upon receptors in the VTA, NA and VP alter behavior. For example, DA injected directly into the NA (Pijnenberg and van Rossum, 1973; Gong et al., 1999) or the VP (Klitenick et al., 1992) increases locomotion. In the NA, this effect is due to activation of D1- like and D2-like receptors (Gong et al., 1999). In the VP this DA-induced locomotion appears to be driven by D1-like receptors and not by activation of D2-like receptors. When injected directly into the VP, quinpirole, a D2R-preferring agonist, attenuates locomotion and SKF38393, a D1R-preferring agonist increases locomotion (Gong et al., 1999).

The exact underlying mechanism by which DA-induced locomotion occurs in the NA-VP pathway is unknown, but it is undoubtedly complex. Electrophysiological and behavioral studies reveal that DA in the NA can decrease the GABAergic drive to the VP, leading to a disinhibition of VP neuronal activity and consequently increases in reward-mediated behavioral activity (Salamone, 1992; Mogenson and Nielsen, 1983; Yang and Mogenson, 1989). Increase in VP activity would result in more GABA released into motor outputs structures, such as the SNr. There is a link between enhanced motor activity and GABA in the SNr. For example, infusions of GABAA agonist muscimol into the SNr increases locomotion (Trevitt et al., 2002). In addition, extracellular GABA increases in the SNr during lever pressing tasks (Correa et al., 2003).

Electrophysiological studies demonstrate that electrical stimulation of VTA neurons can indirectly *increase* VP neuronal activity *via* the NA (Yang and Mogenson, 1989; Yim and Mogenson, 1983), whereas stimulation of VTA neurons can directly

evoke (as indicated by short latency responses) a *decrease* VP neuronal activity (although increases are also detected; Maslowski-Cobuzzi and Napier, 1994; Mitrovic and Napier, 2002). When injected directly into the VP, D1R agonist-induced increases in locomotion appear to be the result of decreases in VP neuronal firing rates. Napier and colleagues demonstrated that local injections of SKF38393 in the VP produce mostly rate decreases in VP neuronal activity whereas quinpirole, an agonist that decreases locomotion when injected directly into the VP, produces mostly increases (Napier and Maslowski-Cobuzzi, 1994). Collectively, the above studies suggest that changes in locomotion are not easily predicted by general increases or decrease in VP neuronal activity.

To add to the complexity of DA-induced effects on the NA-VA pathway, there is evidence that the effects of locally applied DA agonists into the NA and the subsequent effect on firing rates in the VP (as described above) may be different when the DA agonists are systemically administered. For example, evidence suggests that when D1-like or D2-like agonists are systemically injected, the ability of these agonists to induce chances in VP firing rate are not necessarily mediated by the NA. Napier reported that inactivation of the NA with local injection of lidocaine did not influence the firing rate of VP neurons in response to systemic injection of SKF38393, quinpirole or apomorphine (a mixed D1/D2R agonist; Napier, 1992). As these electrophysiological studies were conducted in anesthetized rats, it is of interest to determine if similar results are observed in awake-behaving rats, particularly when reward based learning is occurring.

Electrophysiological studies reveal that systemically administered PPX is able to alter the firing rate of the NA, VP and VTA; studies involving local injection of PPX into these three regions are lacking. PPX is reported to predominantly inhibit neurons in the

NA, but rate-enhancements as well as no effect on firing rate occurred (Piercey, 1998). Our electrophysiological studies indicate that PPX produces equal numbers of rate increases and decreases in approximately half the VP neurons tested. Acute administration of PPX inhibits activity of most VTA neurons (Piercey et al., 1996; Chernoloz et al., 2009), however Chernoloz and colleagues found that D2-like autoreceptors are desensitized following two weeks of repeated PPX treatment (Chernoloz et al., 2009). Given the ability of PPX to influence each of these three brain regions, it is not surprising that PPX can also alter motor activity.

Rodent behavioral studies commonly report an inverted "U-shaped" effect on systemically delivered PPX-induced changes in locomotion. When placed in open field locomotor boxes and tested at least 1hr after injection, rats demonstrate hypo-locomotion at lower PPX doses and hyper-locomotion at higher PPX doses (Lagos et al., 1998; Maj et al., 1997; Chang et al., 2011). Regardless of the dose, if locomotion is measured within the first 30 min hypo-locomotion is commonly observed (Kitagawa et al., 2009; Lagos et al., 1998; Svensson et al., 1994; Chang et al., 2011). When rats are performing operant tasks, published data indicate that PPX, in general, does not impair locomotion, in so much as the rats can perform the operant task (Rokosik and Napier, 2011; Engeln et al., 2012; Collins et al., 2011a; Johnson et al., 2011). However, there were cases in our probability discounting studies, particularly in the sham controls, in which acute administration of PPX did appear to impair motor function. Why motor impairments are expressed in some rats, but not others is not understood, but the Napier lab is continuing to investigate this matter. Tolerance does seem to develop to the motoric effects of acute PPX. We have verified that after six treatments, the motor function of rats was

indistinguishable from that observed during pretreatment baseline, and the rats were able to obtain reinforceres (i.e., electrical brain stimulation) that often superseded baseline (Rokosik and Napier, 2012).

Collectively, data described above indicate that DA, acting on D1-like and D2-like receptors in the VTA-NA-VP pathway influences reward-motivated locomotion; however although PPX can influence general motor activity, studies have not clearly addressed the ability of PPX to influence reward-mediated motor activity. Below are my predictions on the influence that PPX will have on the VTA-NA-VP pathway and subsequent changes in locomotor activity that may occur, particularly when detection of reward prediction errors occurs.

First, in the simplest context, reward-mediated behaviors should be accompanied by the motivation to obtain the reward, thus DA is predicted to be released from the VTA. DA released into the NA and VP should drive locomotor activity (Pijnenberg and van Rossum, 1973; Gong et al., 1999; Klitenick et al., 1992). However, in the presence of PPX, VTA autoreceptor activation would be predicted to attenuate the amount of DA being released. Interestingly, microdialysis studies reveal that DA release in the striatum is not altered during the first two hours following 0.03 or 0.5mg/kg PPX (Lagos et al., 1998). Piercey and colleagues (1996) demonstrated that the firing rate of VTA neurons was attenuated by approximately 50% of baseline following 0.03 mg/kg PPX (iv); 0.3mg/kg PPX attenuated firing rate by 80% of baseline. PPX at a dose of 0.5mg/kg was not tested, but given the dose-related response, it is predicted to nearly silence the neurons. It is predicted then that higher doses of PPX would likely decrease release of DA from VTA neurons, thus decreasing locomotor-inducing effects (Lagos et al., 1998;

Maj et al., 1997; Chang et al., 2011). This attenuation may be overcome by PPX activating postsynaptic D2-like receptors in the NA that should promote locomotion. PPX activating postsynaptic D2-like receptors in the VP may attenuate this predicted outcome of enhanced locomotion, since intra-VP injections of D2-like receptor agonists like quinpirole decreases locomotion (Gong et al., 1999). However, the NA has a higher expression of D3Rs compared to the VP (Bouthenet et al., 1991; Diaz et al., 1995; Sokoloff et al., 1990) and our electrophysiological studies along with data from the Piercey lab (Piercey et al., 1996) indicate that more NA neurons respond to PPX compared to the VP, I predict that in the presence of higher doses of PPX, and in response to the detection of a reward, locomotor activity will be increased.

In situations where there is a positive reward prediction error, the unpredicted reward would be expected to result in phasic release of DA into the NA and the VP. This will disinhibit accumbal GABAergic activity in the VP leading to increase VP neuronal activity and generation of locomotor activity (Salamone, 1992; Mogenson and Nielsen, 1983; Yang and Mogenson, 1989). DA released into the VP would lead to both decreases in firing rates *via* activation of D1Rs, and increases in firing rates in VP neurons, *via* activation of D2Rs (Napier and Maslowski, 1994). The net effect of DA released into the VP would be generation of locomotor activity (Klitenick et al., 1992). However, it is predicted that PPX would i) activate midbrain dopaminergic autoreceptors and decrease the number of neurons able to generate phasic bursts in response the unpredictable reward, thus attenuating locomotion, ii) activate postsynaptic D2-like receptors in the NA which would enhance locomotion, and iii) activate postsynaptic D2-like receptors in the VP which would attenuate locomotion. In this situation, it is

possible that an enhancement in locomotor activity would occur but it may not be specific to the unpredicted reward. Thus, reinforcement learning may be compromised. Following chronic PPX, VTA dopaminergic autoreceptors are desensitized (Chernoloz et al., 2009) and therefore, I predict there may be enhanced activation of behavior in response to unpredicted rewards and potentially enhanced reinforcement learning.

In situations where a negative prediction error occurs, the transient decrease in DA release, followed by subsequent decrease in tonic DA receptor activation in the NA would increase the release of GABA into the VP. This inhibition of VP neuronal function could disengage reward-mediated locomotion. Results from our electrophysiology data suggest that D2/D3Rs are tonically active in the VP; the influence of this on locomotion is unknown. It is possible that it aids to inhibit locomotor activity, if so, a negative prediction error may disinhibit this brake on motor activity. Nevertheless, in the presence of PPX, it is predicted that PPX would activate postsynaptic D2-like receptors in the NA to increase locomotion but also activate postsynaptic D2-like receptors in the VP to decrease locomotion (Gong et al., 1999). In this situation, the reward-related dip in NA and VP DA would be blocked by PPX. The continual stimulation of D2-like receptors in the NA by PPX would be expected to disinhibit VP neuronal activity, although studies in anesthetized rats suggest this may not occur (Napier, 1992). Following chronic PPX, the accumbal DA dip would still go undetected and reward-seeking behavior would persist.

In summary, PPX is expected to enhance locomotor activity generated by the VTA-NA-VP-motor output circuit during positive prediction errors. PPX is also

expected to interfere with learning about reward omissions. Thus, the seeking of rewards would continue even when the rewards are no longer predictable.

Chronic effects of PPX: sensitizing the limbic system

Although acute administration of PPX can alter decision making in many individuals tested in laboratory conditions (Ye et al., 2011; Bodi et al., 2009; Riba et al., 2008; Pizzagalli et al., 2008), only a small (but significant) group of PD patients develop complex ICDs while chronically being treated with PPX. As ICDs develop after continued medication use, adaptations within the system are engaged and thus, other mechanism must be driving ICDs. Converging evidence does suggest that PPX can sensitize the mesolimbic system. For example, studies show that compared to PD patients without ICDs, patients with medication-induced ICDs have a larger release of ventral striatal DA during a gambling task (Steeves et al., 2009) or upon presentation of reward related cue (O'Sullivan et al., 2011). Enhancement of DA release in the ventral striatum has been associated with enhanced salience of drugs (Berke and Hyman, 2000). Sensitization of the mesolimbic pathway is thought to motivate seeking behavior for the drugs and their associated cues (Robinson and Berridge, 2000). Moreover, a novelty-seeking personality has been linked with increased vulnerability to sensitization to psychostimulant-induced DA release in the ventral striatum (Boileau et al., 2006). A study by Bodi and colleagues suggests that PPX increases novelty seeking in PD patients. They found that novelty seeking was low in PD patients before initiation of PPX and three month later it was significantly increased (Bodi et al., 2009). Collectively, these

findings support the possibility that PPX is driving ICDs by sensitizing the mesolimbic reward system and thus enhancing the motivation to seek out rewards.

A role for the VP in PPX-induced ICDs

Our data show that chronic treatment with PPX influences probabilistic learning in a discounting task such that rats are more risky compared to acute administration (Rokosik and Napier, 2012). We also demonstrated this same dosing regimen enhanced the potency of PPX to alter VP neuronal firing rate. Studies by Berridge and colleagues have shown that when the mesolimbic system is sensitized, VP neuronal firing computations shift in a manner that amplifies incentive salience coding of reward-related cues (Tindell et al., 2005). In rats, PPX enhances the salience of cocaine-associated cues (Collins et al., 2011). The motivation to seek out rewards has been related to enhanced salience of rewards and their cues (Robinson and Berridge, 2003). PPX also induces a conditioned place preference indicating that the environmental cues associated with the rewarding effects of PPX gained salience and thus motivated the rat to seek them out (Riddle et al., 2012). We propose that the VP plays a role in mediating these PPX-induced responses such that in the presence of PPX, the VP neuronal activity attributes greater salience to the cues, and in turn motivates the individual to seek out the reward.

Enhanced salience of reward-related cues may also be driven from information processed by other limbic structures. In cases of PPX-induced suppression of VP neuronal activity, more VTA dopaminergic neurons would be spontaneously firing, and thus will be able to respond to rewards and their reward-related cues. This would lead to greater phasic release of DA in the NA which would enhance the incentive salience of the

rewards and cues. Moreover, phasic DA release acting on D1R would facilitate hippocampal inputs to the NA allowing the conditioned environmental cues to drive the motivation of the individual. The VP also projects to several limbic brain regions that mediated impulsivity (see Fig. 3 and 4), including that AMG, NA, PFC, STN and PPN. Therefore the ability of PPX to alter VP neuronal activity will lead to alterations by which these regions integrate reward-related information.

Part of the mechanism by which PPX can influence motivational salience of rewards and reward-related cues is *via* the activation of D3Rs. Involvement of D3Rs in reward-seeking behavior has been established in animal studies. These experiments demonstrate that antagonism of D3Rs attenuate actions of several abused drugs in various rodent models of drug addiction (for review, see (Heidbreder and Newman, 2010), including drug- cue- and stress-induced reinstatement (Xi and Gardner, 2007; Vengeliene et al., 2006; Xi et al., 2006; Gilbert et al., 2005; Xi et al., 2004). A recent clinical study reported that pathological gamblers were found to have a positive correlation between D3R levels and gambling severity and impulsiveness (Boileau et al., 2012). In our studies, we found that D3Rs activated by PPX are sufficient to alter the neuronal firing of VP neurons. We propose the mechanism by which chronic PPX treatment can enhance the ability of PPX to alter VP firing rate involves an upregulation of D3R function that drives reward-seeking behavior.

The PD brain state as a vulnerability to develop ICDs

There are several reported vulnerabilities found in the subpopulation of PD patients that develop PPX-induced ICDs. These include male sex, younger age, younger

age at PD onset, a pre-PD history of ICD(s), personal or family history of substance abuse, gambling problems, and impulsive personality traits (Voon et al., 2007; Weintraub et al., 2010). However, few laboratories have addressed the possibility of a PD-like brain state as a vulnerability factor. Prevalence studies report approximately 14% of PD patients develop ICDs (Weintraub et al., 2010). This is in contrast to approximately 5% of RLS patients (Voon et al., 2011b). However, it should be noted that epidemiological studies in RLS patients is have not been as extensive as those conducted in the PD population. We explored the differences between PD-like rats and controls in this dissertation.

In our behavioral studies, we found that, at least at the 2mg/kg dose of (±)PPX tested, partial striatal lesions targeted to the DLS do not influence the ability of PPX to enhance probability discounting. As smaller doses of PPX can influence discounting behavior (Madden et al., 2010), future studies should repeat these experiments with smaller doses of PPX to determine if PD-like rats are more sensitive PPX-induced increases in probability discounting.

In our electrophysiological studies, this same 6-OHDA-induced DLS lesion had no influence on PPX-induced alterations in VP neuronal firing rates. However, there was evidence for a trend in enhanced potency of acute (-)PPX in rats with a more complete lesion to the ascending dopaminergic pathway that included the SN/VTA. I propose that the relevance of this latter finding may lie in the ability of PPX to effectively treat depression in patients with PD (Seppi et al., 2011) rather than influence impulsivity. A role for the VP in depressive–like behaviors is emerging. For example, learned

helplessness, anhedonia (Skirzewski et al., 2011), and effort related behaviors (Farrar et al., 2008) have been linked to the VP.

Two other studies have investigated the ability of the PD-like brain state to alter rewarding behavioral outcomes associated with PPX administration. Both studies focused on the intrinsic rewarding properties of PPX and used rat models of early stage PD. The Napier lab recently demonstrated that PPX can induce a conditioned place preference in rats with partial striatal lesions targeted to the DLS as well as vehicle-DLS treated control rats; however, a higher dose of PPX was needed to induce a place preference in control rats compared to PD-like rats (Riddle et al., 2012). On the other hand, Engeln and colleagues found no differences in the ability of PD-like rats and controls to self-administered PPX (Engeln et al., 2012). Clearly, further studies are necessary to assess the PD-like brain state in animal models. Investigation should include assessments with a range of PPX doses and as well as other models of PD. It also appears important to differentiate behavioral outcomes based on instrumental learning and associative learning.

Considering only 14% of PD patients develop DA agonist-induced ICDs, this suggests a general PD-like brain state may not be a vulnerability factor. The PD-like brain state is not homogenous across patients; thus, if there is a PD-brain state vulnerability, it may lie in the type of lesion expressed (i.e., location, extent, etc). Rabinak and Nirenberg, 2010 found that PD patients with ICDs had lower unified Parkinson's disease rating scale motor scores than those without ICDs (disease duration and total DA replacement therapy use were similar). The authors suggest that patients with ICDs may have a more preserved nigrostriatal dopaminergic function (Rabinak and

Nirenberg, 2010). To further investigate this, clinical investigations into the similarities of the pathological brain state of those that develop ICDs *vs.* those that do not would help to elucidate what aspects of PD-like brain state renders them more sensitive.

Another factor that may play a role in the susceptibility of ICDs is the expression of D2/D3Rs. For example, lower baseline levels of ventral striatal D2Rs were found in PD patients with pathological gambling compared to control patients (Steeves et al., 2009). Studies in rats (Dalley et al., 2007), primates (Nader et al., 2002; Nader et al., 2006), and humans (Volkow et al., 2002; Volkow et al., 1999) suggest this is an underlying vulnerability to addiction. Genotypes associated with reduced D2Rs in the striatum (Hirvonen et al., 2004) are associated with a reduction in learning from negative consequences (Frank et al., 2007; Frank and Hutchison, 2009; Klein et al., 2007). In PD, there are alterations that occur with D2 and D3Rs, such that the degeneration of the ascending dopaminergic system induces adaptations leading to an increase in D2Rs and a decrease D3Rs (Rinne et al., 1990; Brooks et al., 1992; Ryoo et al., 1998; Boileau et al., 2009; Graham et al., 1990). Preclinical studies support this (Bordet et al., 1997; Stanwood et al., 2000a). Such receptor profiles would indicate non-impulsive personality traits which, in general, are reported in PD patients (Menza et al., 1993;Bodi et al., 2009). However, studies suggest that chronic treatment with dopaminergic drugs produces an upregulation of D3Rs and a downregulation of D2Rs. Indeed, human PET studies have revealed an upregulation of striatal D2R in non-treated PD patients; this upregulation persisted while patients received no antiparkinsonian medication (Rinne et al., 1990); however, there was a relative downregulation of D2Rs in patients on medication (Antonini et al., 1997). In rodents with a unilateral MFB lesion, D3Rs decrease in the

NA 21 days after the lesion while subsequent L-DOPA administration enhances expression above baseline levels (Bordet et al., 1997). Thus, differences in the adaptation processes that occur with D2Rs and D3Rs before and after DA medication may confer differences in the vulnerability to engage in behavioral addictions.

Summary and Conclusions

In conclusion, this dissertation significantly contributes to the field of DA agonist-induced ICDs by developing a preclinical model of PPX-induced risk-taking that can be used to advance our knowledge of the neuropsychopharmacology underlying this phenomenon. Our novel probability discounting paradigm was successful in modeling the clinical scenario in which PPX enhances risk-taking in both healthy controls and PD patients. Importantly, we demonstrated predictive validity with our model as rats displayed more risky behaviors after both acute and chronic PPX treatment, this outcome ceased shortly after PPX treatment was discontinued, and risk-taking was reinstated after treatment re-initiation. There are several important applications of this behavioral paradigm. For example, understanding the contribution of D2/D3Rs in PPX-induced impulsivity is critical for future development of compounds therapeutic for movement disorders but devoid of inducing impulsive behaviors. Thus, future studies can elucidate the underlying pharmacology of PPX-induced risk-taking by using D2R- and D3R-selective antagonists in our paradigm. In addition, the most effective way to currently manage DA agonist-induced ICDs is to lower the dose or switch to an alternative therapy, both of which may be less effective in treating the targeted neuropathological symptoms. Our behavioral protocol can be used as a tool to screen new DA agonists for their

potential to induce impulsivity and also be used to screen potential therapies for gambling disorders and other behavioral addictions. The behavioral protocols also have the potential to be modified for the study of other aspects of impulsivity including to impulsive choice. In the second part of this dissertation, our electrophysiology data suggest that the VP may be a prominent player in the circuitry responsible for driving impulsivity. Using the ICSS-meditated probability discounting paradigm, future studies can elucidate the role of the VP in PPX-induced risky behavior by determining if the VP is necessary or sufficient for this outcome. Based on our electrophysiology data, we propose that one mechanism by which PPX may influence decision making in individuals with ICDs is *via* an increase in limbic D3Rs that, when activated by PPX, alters VP neuronal function. In the presence of reward-related cues, the sensitized response of VP neurons attributes greater salience to these cues. This drives the motivation to engage in the behavioral addictions. Moreover, in the case of pathological gambling, patients that are on their PPX medication will continue to show lack of self-control and engage in behaviors that are risky and uncertain because the prospect of winning, rather than the fact that negative outcomes are accumulating, is driving their decision-making process.

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VITA

Sandra was born and raised in Chicago, Illinois. After graduating from Queen of Peace High School in 1997, she attended Florida International University on a Division I volleyball scholarship. After two years, she transferred to Illinois State University and in 2002 she graduated summa cum laude with a dual Bachelor's degree in Biology and Psychology. During her time at ISU, she worked in both an ecology lab, under Dr. Steven Juliano and a neurobiology lab under Dr. Paul Garris.

After two years of working as a research technician in the neurology laboratory of Dr. Un Kang at the University of Chicago, Sandra joined the Neuroscience Program at Loyola University Medical Center. Sandra has completed her doctoral studies under the guidance of Dr. T. Celeste Napier. Sandra's research explored the behavioral and electrophysiological effects of pramipexole. This dopamine agonist is used for therapy in Parkinson's disease as well as restless leg syndrome, and it is also able to induce impulsivity in some patients. Sandra has presented several posters and has published four first author papers. In 2010, she won both a travel award and most outstanding poster at the Behavior, Biology and Chemistry Conference held in San Antonio, Texas.

Sandra is currently a post-doctoral fellow at the Edward Hines Jr. VA Hospital. She is working at the Center for Management of Complex Chronic Care in the Health Services Research and Development Care Department. Her primary mentor is Dr. Theresa Pape and her secondary mentor is Dr. Francis Weaver. Sandra's research is

focusing on the development of therapies for cognitive impairment in Parkinson’s disease patients.