THE BEHAVIORAL AND NEUROPHYSIOLOGIC EFFECTS OF ACUTE DOPAMINE RECEPTOR BLOCKADE IN THE MACAQUE STRIATUM

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The pathophysiology of Parkinson's disease (PD) has long been attributed to dopamine (DA) loss in the striatum. However, it remains unclear whether simple underactivation of striatal DA receptors is sufficient to induce parkinsonian signs. To test this hypothesis, we performed unilateral infusions of cis-flupenthixol (cis-flu; D1/D2 antagonist) into the macaque putamen, while the macaque performed a reaching task. Twenty-six cis-flu and three saline infusions were performed across three hemispheres in two macaques. Neuronal and local field potential activity was recorded simultaneously from cortex, globus pallidus externa (GPe), and globus pallidus interna (GPi) during most infusions. The reaching task required each macaque to make visually-cued reaching movements to a target for a reward. The macaque was then required to return its hand to a home position without external cues. Injection-related slowing of movement initiation or execution was thought to reflect akinetic- or bradykinetic-like effects, respectively. Following 8/26 cis-flu infusions, macaques exhibited a marked slowing in the initiation of self-generated return movements (95% increase). This was the most severe behavioral effect of cis-flu infusions. The initiation and execution of externally-cued movements were also prolonged following 9/26 and 6/26 injections, but only by 20% and 15% respectively. In general, akinetic-like effects occurred twice as often as bradykinetic-like effects ($p<0.05$, $\chi^2= 4.1$). Interestingly, akinetic and bradykinetic effects could be elicited independently. In addition to affecting behavior, intrastriatal DA receptor blockade also reduced resting and peri-movement activity in

the cortex and suppressed resting GPe activity. Burstiness, synchrony, and oscillatory activity in cortex were increased following intrastriatal DA receptor blockade as well. Oscillatory activity was also increased in the GPe and GPi. In conclusion, suppression of striatal DA activity was sufficient to induce akinetic-like signs, most severely affecting movement initiation during self-generated movements. Furthermore, distinct parkinsonian-like signs could be elicited independently, suggesting that separate signs may have unique pathophysiologic substrates. Intrastriatal DA receptor blockade also induced changes in cortical and BG activity that were consistent with findings in the parkinsonian state. Interestingly, many of these neuronal activity changes were specific to cortex, implicating an important role for cortical activity in the development of akinetic parkinsonian signs.

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PREFACE

This research was supported by the National Institute of Neurological Disorders and Stroke at the National Institutes of Health, grant number R21-NS55197 to RST, by the National Institutes of Health, grant number T32 NS007433 in support of Vanessa Chan, and by the Clinical and Translational Science Institute Multidisciplinary Predoctoral Fellowship program, awarded through the Clinical and Translational Science Institute and the Institute for Clinical Research Education at the University of Pittsburgh, grant 5TL1RR024155-04 in support of Vanessa Chan.

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1.0 INTRODUCTION

Parkinson's disease (PD) patients exhibit a multi-faceted symptomatic profile, primarily characterized by rigidity, tremor, bradykinesia (slowness of movement), and akinesia (difficulty initiating movement). Many PD patients suffer from cognitive, autonomic, and postural deficits as well. Despite such a diverse symptomatic profile, PD has long been attributed to one simple cause: loss of dopamine (DA) in the striatum (Kish et al., 1988). Striatal DA loss is thought to induce the signs of parkinsonism by setting off a cascade of firing rate changes throughout the basal ganglia (BG), culminating in a suppression of motor cortical activity that is thought to impair movement (Albin et al., 1989; DeLong, 1990; Wichmann and DeLong, 1996). Emerging evidence, however, suggests that abnormal neuronal activity patterns in the BG and cortex may be more important in the pathophysiology of PD than changes in overall firing rates (see *Section 1.3: Pathophysiology of PD*). It therefore remains unclear whether striatal DA loss is sufficient to alter neuronal firing in the BG or the cortex, or even induce parkinsonian signs. Various other pathologic changes that occur in the parkinsonian state may play a more important role in the development of abnormal neuronal activity patterns and specific parkinsonian signs than striatal DA loss (see *Section 1.2.2. Pathology of PD*). Thus, three important questions about the pathophysiology of PD remain unanswered. First, is striatal DA loss sufficient to induce specific parkinsonian signs? Second, is striatal DA loss sufficient to elicit abnormal neuronal activity patterns in the cortex and BG that are associated with the parkinsonian state? Third, are these

changes in neuronal activity related to specific parkinsonian signs? The foundation for these questions has been laid over the last few decades of work, and will now be reviewed in detail.

This introduction begins by describing the anatomy and function of the BG and how it communicates with cortex. The role of DA in the BG is then discussed, followed by an overview of PD, a disease thought to be caused by a loss of DA in the BG. Finally, various hypotheses surrounding the pathophysiology of PD are discussed. This introduction is not only meant to provide the background information necessary to understand the importance of this work, but also to illustrate how far the field has come in pursuit of these questions.

1.1 THE BASAL GANGLIA

1.1.1 The Anatomy of the Basal Ganglia

The BG consists of a group of subcortical nuclei, including the striatum, globus pallidus externa (GPe), globus pallidus interna (GPi), and subthalamic nucleus (STN). The striatum, one of the primary input structures of the BG, is composed of two nuclei: the caudate and putamen. The striatum receives information from the cerebral cortex and thalamus (Alexander et al., 1990; Donoghue and Herkenham, 1986; Kelly and Strick, 2004). Projections to the striatum synapse predominantly on dendritic spines of medium spiny neurons [MSNs; the main output neurons of the striatum (Kemp and Powell, 1971; Sadikot et al., 1992)], but also project to striatal interneurons (Sidibe and Smith, 1999). Dopaminergic projections to the striatum from the substantia nigra pars compacta (SNpc) synapse on the necks of these dendritic spines, so they are ideally situated to regulate the effects of cortical inputs on MSNs (Smith and Bolam, 1990).

All information entering the striatum travels through the direct or indirect pathway (Wichmann and DeLong, 1996). Striatal neurons with D1 receptors transmit information through the direct pathway to the GPi, while neurons with D2 receptors send information through the indirect pathway to the GPe . The GPe then projects to the STN (Nauta and Mehler, 1966). The STN also receives input from the brainstem via the peduncolopontine nucleus (PPN) (Hammond et al., 1983; Jackson and Crossman, 1983; Lavoie and Parent, 1994) and from the cortex via the hyperdirect pathway (Nambu et al., 2002b). Information from the STN projects to the GPi before exiting the BG (Nauta and Cole, 1978). The GPi serves as the primary motor output of the BG, projecting information back to the cortex through the thalamus (Nauta and Mehler, 1966; for review, see Wichmann and DeLong, 1996) (See Figure 1). There is recent evidence, however, that the STN also projects directly to the thalamus (Rico et al., 2010) and di-synaptically to the cerebellum (Bostan et al., 2010), and may therefore represent another major output nucleus of the BG.

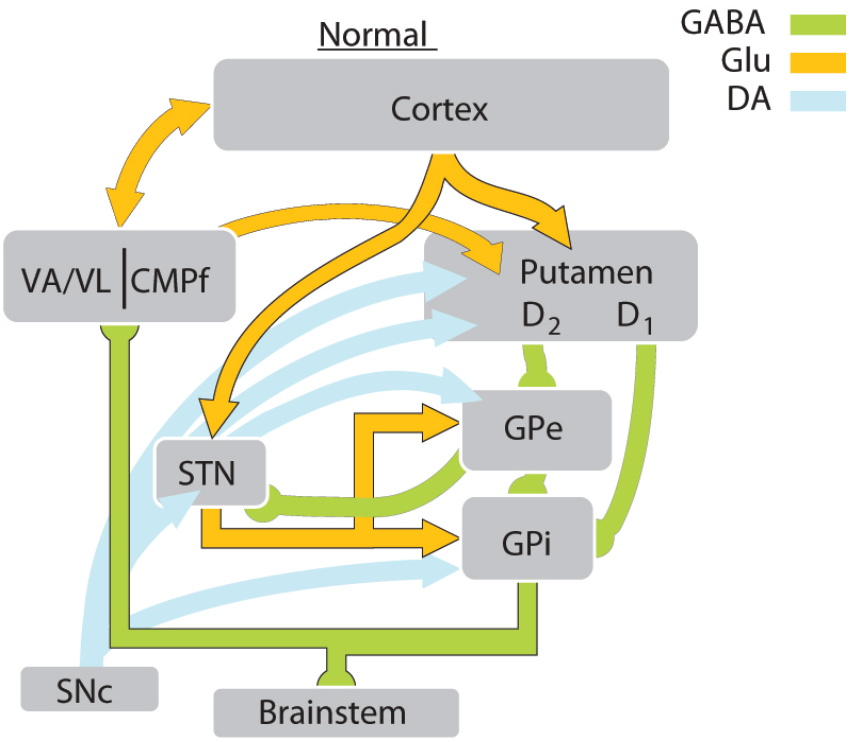

Figure 1: Wiring diagram of the BG

This figure was reproduced from with permission from the author (Turner, 2009).

The information transmitted through cortex, BG, and thalamus is organized into functionally-segregated loops, such that separate circuits that loop between cortex and BG carry specific associative, motor, or limbic information (Alexander et al., 1990; DeLong et al., 1985). Limb- and task-specific information is also transmitted in parallel cortico-BG-thalamocortical loops, such that distinct areas of primary motor (M1) and premotor cortex (PMC) project to separate sub-regions of the BG (Selemon and Goldman-Rakic, 1985; Takada et al., 2001) (See Figure 2). The way in which information is transmitted between BG nuclei through these loops is quite complex.

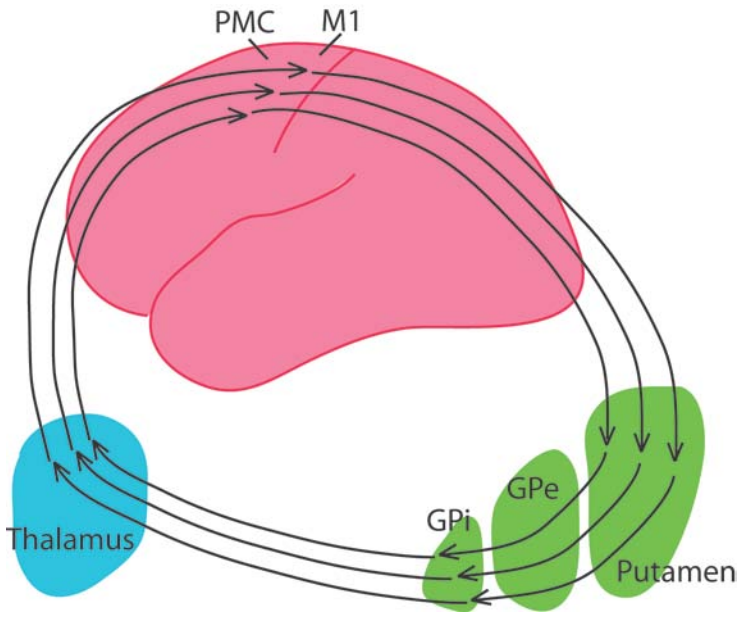

Figure 2: Functionally-segregated cortico-BG-thalamocortical loops

This figure represents a diagram illustrating how distinct areas of M1 and PMC project to separate sub-regions of the BG, which then project back to the same regions of cortex.

1.1.2 Basal Ganglia Neurophysiology

Information is communicated between BG nuclei via one of three pathways: the direct pathway, the indirect pathway, and the hyperdirect pathway. When excitatory cortical inputs excite GABAergic striatal neurons that project to the direct pathway, the activity of GABAergic GPi neurons is suppressed. This is thought to disinhibit glutamatergic thalamic neurons, thereby causing activation of cortical neurons and facilitating movement. Conversely, when GABAergic striatal neurons projecting to the indirect pathway are activated, GABAergic neurons in the GPe are suppressed, causing disinhibition of glutamatergic STN neurons which then activates GABAergic GPi neurons. Consequently, the activity of glutamatergic thalamic neurons is suppressed, causing inhibition of cortical activity and movement repression. In this way, the

direct pathway of the basal ganglia is thought to facilitate movement, while the indirect pathway is believed to suppress movement (See Figure 1). Since DA exerts a net excitatory effect on neurons of the direct pathway and a net inhibitory effect on neurons of the indirect pathway, DA is thought to facilitate movement (for review, see Wichmann and DeLong, 2003). This model of BG function is thought to be overly simplistic, however (for a more detailed discussion of DA, please see *Section 1.1.5. Dopamine*). In fact, the functional role of the BG in movement is widely debated.

1.1.3 The Functional Role of the Basal Ganglia

The BG is thought to play an important role in performing sequences of movements, motor learning, habit formation, generating self-initiated movements, or modulating specific kinematic parameters such as amplitude, velocity, and direction (for review, see Turner and Desmurget, 2010; Wichmann and DeLong, 1996). Some hypothesize that the BG plays an important role in action selection by facilitating some movements while inhibiting others (Mink, 1996). This hypothesis is challenged by the fact that peri-movement changes in GPi activity occur too late to be involved in action selection, often beginning after movement onset (Mink and Thach, 1991a; Turner and Anderson, 1997). Various studies have attempted to study the role of the BG in motor control by determining which behavioral functions are lost by inactivating or silencing the output nucleus of the BG, the GPi.

Inactivation of the sensorimotor area of the GPi does not disrupt the performances of motor sequences or overlearned serial skills, reducing the likelihood that the BG contributes substantially to these functions (Desmurget and Turner, 2008). Instead, suppressing activity in the sensorimotor GPi causes dysmetria and slowed movements (Desmurget and Turner, 2008;

Kato and Kimura, 1992) and a drift in arm position (Inase et al., 1996) in monkeys. Lesioning the GPi also slows movements in monkeys (Horak and Anderson, 1984; Mink and Thach, 1991b). These findings, among others, support the idea that the BG is involved in modulating movement performance in response to motivational states (for review, see Turner and Desmurget, 2010). The functional role of the BG remains poorly understood, however, making it difficult to predict how dysfunction of specific BG nuclei might cause specific movement disorders. Nevertheless, the dysfunction of the striatum has been implicated in various movement disorders.

1.1.4 The Striatum

The striatum is mostly composed of inhibitory, GABAergic medium spiny neurons (Yelnik et al., 1997). The remaining cells include a combination of cholinergic or GABAergic interneurons, some of which express DA receptors (Tepper et al., 2004). As previously mentioned, input from separate cortical areas projects to relatively distinct regions of the striatum. In this way, the striatum is organized into functional territories that are believed to play separate roles in associative, motor, or limbic functioning. Since DA loss in pre-motor and motor sub-regions of the striatum is thought to cause the primary motor signs of PD, I will focus on the anatomy and physiology of the motor and premotor subregions of the striatum here.

Projections from M1 and SMA to the striatum Striatal inputs from the M1 primarily project to the posterolateral putamen. Inputs from hindlimb, forelimb, and orofacial regions of M1 terminate in dorsal, intermediate and ventral sub-sections of the putamen, respectively. In contrast, bilateral inputs from the supplementary motor area (SMA; pre-motor cortical structure) project primarily to the medial putamen (Takada et al., 1998) (See Figure 3). The distinction

between M1- and SMA-receiving territories of the striatum was observed in neurophysiologic studies (Takada et al., 1998) and verified using antidromic stimulation (Nambu et al., 2002a). There is evidence, however, that the segregation of inputs from SMA and M1 to the striatum may not be absolute.

Projections from M1 and SMA were found to overlap partially in the mediolateral central putamen (Takada et al., 1998). Furthermore, twenty percent of cells in the intermediate zone of the striatum responded to stimulation in both M1 and SMA (Nambu et al., 2002a). These reports are consistent with the idea that some cells receive inputs from both M1 and SMA; however, there is evidence that projections from M1 and SMA interdigitate, rather than intermingle, in this intermediate zone (Selemon and Goldman-Rakic, 1985). Taken together, these findings suggest that mediolateral central regions of the putamen receive projections from both M1 and SMA (See Figure 3). It remains unclear whether inputs from M1 and SMA project to the same individual cells.

Projections from PMd to the striatum Bilateral inputs from dorsal and ventral premotor cortices (PMv and PMd; pre-motor cortical structures) project to the dorsomedial striatum (See Figure 3). These projections partly overlap with SMA projections, but not with those from M1 (Takada et al., 1998). Inputs from both SMA and PMd are also found in striatal bridges linking the caudate to the putamen, as well as the lateral caudate (Takada et al., 1998). More anterior regions of the putamen receive inputs from the SMA, pre-SMA, and dorsal cingulate motor cortex (Takada et al., 2001). In summary, inputs to the striatum from separate motor and premotor cortical regions terminate in relatively segregated regions, but there is evidence of some overlap between inputs from SMA and M1, as well as SMA and PMd (See Figure 3). Here, I propose to test the hypothesis that DA loss in these premotor and motor areas of the

putamen is sufficient to induce parkinsonian motor signs. Furthermore, I will determine whether blocking DA activity in separate striatal subregions is sufficient to induce specific signs of parkinsonism. Possibly, DA loss in pre-motor or motor territories could selectively impair movement initiation or execution, respectively. Before we can hypothesize how striatal DA loss might affect specific behaviors, it is first necessary to understand the basic neurophysiology of the striatum, and the important role of striatal DA.

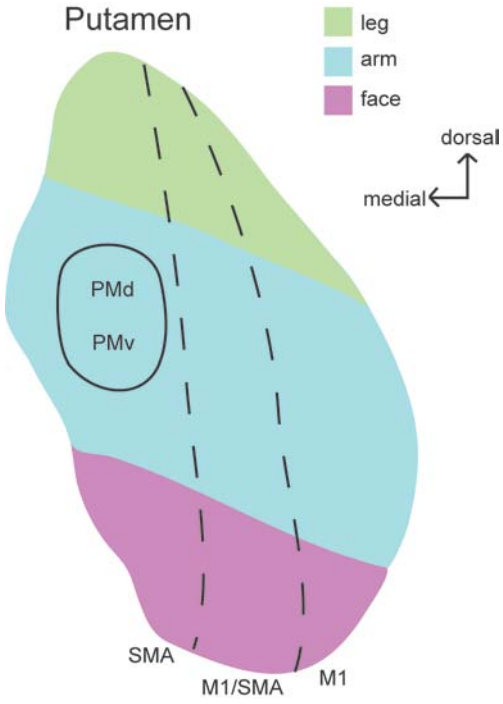

Figure 3: Premotor and motor subdivisions of the putamen

This figure represents a schematic of the anatomical projections to the putamen, as depicted from a coronal view. The most medial territory receives inputs from SMA, PMd, and PMv, thereby receiving only premotor input. The central region receives inputs from M1 and SMA, thereby receiving both motor and pre-motor input. The most lateral region receives inputs from M1 and is therefore deemed the motor territory.

1.1.5 The neurophysiology of the striatum

Typically, MSNs remain in a "down" state, with a resting membrane potential of approximately

85 mV, due to the dominant activity of K+ channels**.** Striatal cells can transition to an up state in response to temporally convergent excitatory synaptic cortical inputs (Wilson, 1993). Only in the up state, MSNs can fire action potentials in response to further depolarization. MSNs are therefore relatively inactive much of the time, firing only in response to strong inputs (Wilson, 1990). Their activity is also influenced by striatal interneurons. Striatal interneurons are mostly GABAergic. They are typically inactive, with the exception of cholinergic tonically active units (TANs) (Wilson, 1990). Like MSNs, interneurons express DA receptors (Lehmann and Langer, 1983), providing another mechanism whereby striatal DA levels can impact MSN activity. While most striatal cells are inactive much of the time, imaging (Cunnington et al., 2002; Francois-Brosseau et al., 2009) and electrophysiologic (Alexander and Crutcher, 1990; Kimura et al., 1992; Lee and Assad, 2003; Romo et al., 1992; Schultz and Romo, 1992) studies demonstrate that the putamen is activated both during preparation and execution of movements.

Movement-related activity The posterolateral putamen is most commonly recognized as the "motor" region of the striatum due to the aforementioned projections from M1 to this region and the movement-related activity of MSNs in this region. Cells with movement-related activity are also found in the anterior striatum, however (Romo et al., 1992). This movement-related activity is typically not very selective for self-initiated or externally-cued movements. Interestingly, some MSNs exhibit a combination of movement-related and preparatory activity (Kimura et al., 1992).

Preparatory activity Most MSNs with preparatory activity are found more rostral and medial than those with movement-related activity (Alexander and Crutcher, 1990). Throughout the anterior striatum, cells with preparatory activity fire selectively for the preparation of externally-cued or self-initiated movements (Schultz and Romo, 1992). The activity of cells that

fire selectively before self-initiated movements typically begins 0.5-5 seconds before movement onset, ramps up slowly, and peaks around the time of movement onset before subsiding again. A similar type of slowly-ramping activity during the preparation of self-initiated movements is also found in the posterior putamen and the SMA (Romo and Schultz, 1992). This slowly increasing activity in the striatum and SMA may represent successive reverberations of activity through BG-thalamocortical loops. Schultz and Romo (1992; 1992) have postulated that a self-generated movement is initiated when enough reverberations of this activity generates enough activity to surpass an arbitrary activity threshold. Taking this hypothesis one step further, Lee and Assad (2003) suggested that striatal DA loss in PD may slow the initiation of self-generated movements by reducing the excitability of striatal neurons, thereby attenuating the rise of activity to threshold.

In contrast to the slowly ramping preparatory activity observed before self-generated movements, MSNs exhibit sharp, rapid increases in activity before externally-cued movements. In the DA-depleted state, therefore, attenuated activity during the preparation of self-initiated movements could be elevated above threshold in response to an external cue. This represents one theoretical mechanism whereby PD patients might overcome akinetic episodes using external cues (Lee and Assad, 2003). In order to explore all of these interesting hypotheses further, it is first necessary to understand the role of DA in the striatum.

1.1.6 Dopamine

Dopaminergic Neurons Dopaminergic input to the striatum arises from projection neurons in the SNpc and ventral tegmental area (VTA) (Graybiel et al., 1990; Lavoie et al., 1989). These projection neurons also send collaterals to the GPe, GPi, STN, thalamus, M1, and PMC,

providing a source of DA to these regions (Cossette et al., 1999; Deniau et al., 1980; Fallon, 1981; Fallon and Loughlin, 1982; Francois et al., 2000; Freeman et al., 2001; Hedreen, 1999; Lindvall and Bjorklund, 1979; Loughlin and Fallon, 1984; Nobin and Bjorklund, 1973; Sanchez-Gonzalez et al., 2005; Takada and Hattori, 1986).

The activity of DA neurons is thought to encode reward signals (Romo and Schultz, 1990) and reward prediction error (Schultz et al., 1997), or the difference between the prediction and existence of a reward. More specifically, unpredicted rewards increase the activity of dopaminergic neurons, while predicted rewards elicit no change in dopaminergic cell activity (Schultz et al., 1997). When predicted rewards fail to occur, the activity of dopaminergic neurons is suppressed. In this way, dopaminergic signaling is thought to reinforce rewarding behaviors and facilitate learning (for review, see Schultz, 1998).

The role of striatal DA cannot simply be inferred from the activity of dopaminergic neurons, however, because the activity of dopaminergic neurons is not directly related to modulations in extrasynaptic striatal DA (for review, see Schultz, 1998). The nonlinear relationship between the activity of DA neurons and fluctuations in extrasynaptic striatal DA levels is attributable to the following factors: the activity of dopamine reuptake transporters, presynaptic modulation of neuronal firing, changes in tonic firing rates of SNpc cells, variation in the number of DA reuptake sites throughout the striatum, and the duration and timing of DA release. Thus, while the activity of dopaminergic neurons certainly contributes to striatal DA activity, fluctuations in striatal DA are largely governed by other processes as well. The functional purpose of the resulting fluctuations in striatal DA remains unclear.

Function of DA Striatal DA is necessary for long-term potentiation (LTP) and long-term depression (LTD) at corticostriatal synapses, important processes in plasticity and learning

(Calabresi et al., 2007; Gerdeman et al., 2002; Kreitzer and Malenka, 2007; Reynolds et al., 2001). The effects of DA on synaptic plasticity are thought to contribute to habit formation, long-term storage, and the retrieval of well-learned movements, especially when learning is associated with rewarding stimuli (Beninger et al., 1993; Nakamura and Hikosaka, 2006; Reynolds et al., 2001). Striatal DA is therefore thought to be important in numerous aspects of behavior. The actions of DA vary depending on the specific DA receptors that are bound.

Striatal DA receptors There are five distinct DA receptors grouped into two classes: D1-receptors (D1 and D5) and D2- receptors (D2, D3, D4). In the striatum, D1-receptors are only found on postsynaptic neurons, while D2-receptors are found both presynaptically and postsynaptically (Rankin M.L., 2010; Rondou et al.). Presynaptic striatal D2-receptors provide negative feedback to presynaptic neurons and can adjust these cells' firing rate, synthesis, and release of DA (Missale et al., 1998; Sibley, 1999; Wolf and Roth, 1990). The activity of presynaptic DA receptors can also modulate the activity of corticostriatal neurons (Garcia-Munoz et al., 1991). Specifically, DA agonists were found to depress the excitability of corticostriatal neurons, while DA antagonists abolished this effect. In contrast to presynaptic receptors, postsynaptic striatal D2-receptors are only thought to act on postsynaptic neurons.

As previously mentioned, activating D1 receptors on MSNs of the direct pathway is thought to suppress GPi activity, while D2 receptor activation on MSNs of the indirect pathway is believed to increase GPe activity, which decreases GPi activity. This model of dopaminergic function in the striatum is quite simplistic, however. Recent evidence suggests that the effects of D1- and D2- receptor activation on striatal MSN activity is complex and depends on the state of the neuron.

D1 receptors are activated during transient bursts of dopaminergic cell activity (Surmeier

et al., 2007). In the down state, activation of D1 receptors enhances the activity of potassium channels and reversibly inhibits the activity of sodium channels, making it difficult for the cell to fire. In the up state, D1 receptor stimulation increases the likelihood that the cell will fire by enhancing the activity of L-type calcium channels (Hernandez-Lopez et al., 1997). This voltage-dependent activity of D1 receptors is thought to reduce noise by suppressing responses to weak excitatory synaptic input in the down state while enhancing evoked activity in the up state (Pierce and Rebec, 1995; Rolls et al., 1984). Indeed, DA iontophoresis on MSNs causes a net excitation of movement-related cells relative to cells without movement-related activity.

Due to the higher affinity of D2 receptors for DA, basal levels of striatal DA primarily activate D2 receptors on striato-GPe MSNs. Stimulation of D2 receptors suppresses L-type Ca channels, enhances potassium channel activity (Greif et al., 1995; Hernandez-Lopez et al., 2000; Inase et al., 1996) and suppresses sodium currents (Surmeier et al., 1992). This reduces the responsiveness of MSNs to inputs, independently of the cell's state.

D1 and D2 both play an important role in modulating plasticity at corticostriatal synapses through LTP and LTD. Thus, DA can alter the efficacy of cortico-striatal synaptic connections, changing the response of striatal neurons to cortical inputs in an activity-dependent manner (Wickens et al., 1996). D1-receptor activation is thought to facilitate LTP (Calabresi et al., 2007; Yin and Knowlton, 2006) while D2-receptor activation is believed to promote LTD (Gerdeman et al., 2002; Kreitzer and Malenka, 2007). Following striatal DA loss, however, cells with D2 receptors exhibit LTP in situations that would normally induce LTD (Shen et al., 2008). Similarly, cells with D1 receptors exhibit LTD in situations that would normally induce LTP. Thus, striatal DA loss may disrupt the balance of activity between the direct and indirect pathway.

Extrastriatal DA Outside of the striatum, DA is thought to influence motor control at various sites. Evidence for this comes from the findings that intra-GPe and intra-STN DA receptor blockade induce catalepsy in rodents (Costall et al., 1972; Hauber, 1998). These results are difficult to interpret, however, as there is no clear parallel of rodent catalepsy in human behavior. The catelptic state is characterized by a nonspecific combination of abnormal posturing and immobility. It therefore remains unclear which specific aspects of motor control were impaired by intra-GPe and intra-STN DA receptor blockade. This makes it challenging to define a potential role of extrastriatal DA loss in the development of specific parkinsonian signs. Future studies that can tease apart more specific behavioral effects of blocking DA activity in the striatum, GPe, GPi, and STN are necessary. Such studies would allow us to determine how striatal and extrastriatal DA loss may contribute to the development of specific parkinsonian signs. Here, I will suppress DA activity in the putamen of macaques while they perform a reaching task that permits the independent study of the initiation and execution of movements. In this way, I will determine the specific behavioral effects of striatal DA loss alone.

1.2 PARKINSON'S DISEASE OVERVIEW

Striatal DA loss is thought to induce parkinsonian signs by causing BG dysfunction. Indeed, a substantial amount of striatal DA loss is a pathologic hallmark of PD (Kish et al., 1988). Furthermore, neuronal activity in the BG becomes abnormal in the parkinsonian state (see *Section 1.3: Pathophysiology of PD*). Finally, lesions of the output nuclei of the BG dramatically reduce the severity of parkinsonian signs (Coban et al., 2009; de Bie et al., 1999; Vitek et al., 2003). All of these findings support the idea that BG dysfunction due to striatal DA

loss causes PD. However, we remain unable to delineate which of the various pathologic findings in PD patients cause the development of parkinsonian signs and which may simply be associated with PD (for review, see Wichmann and DeLong, 2003). Therefore, the pathophysiology of PD remains unclear and we are left with an ambiguous target for future therapies. Before discussing the primary overarching hypotheses surrounding the pathophysiology of parkinsonian signs in depth, I will provide a general overview of the signs and treatments of PD.

1.2.1 Primary Signs of Parkinson's disease

PD patients exhibit a multi-faceted symptomatic profile, characterized by rigidity, akinesia, bradykinesia, and tremor. Rigidity describes increased muscular resistance to passive joint movement. Frequently occurring with rigidity, akinesia describes an idle state without movement, despite intact motivation and elemental motor functions (Yokochi, 2009). One debilitating manifestation of akinesia involves freezing episodes, or temporary periods of time marked by the complete inability to initiate movements (Fahn, 1995; Jankovic, 2008). Patients can often overcome freezing episodes with the help of external cues (Arias and Cudeiro, 2008; Dietz et al., 1990; Marchese et al., 2000). Generally, in fact, PD patients have more difficulty initiating movements in the absence of external cues (Flowers, 1976; Morris et al., 1996; Oliveira et al., 1997).

While akinesia primarily describes the impaired initiation of movement, bradykinesia refers to the slowed execution of movement. Bradykinesia may be caused by an inability to correctly scale the amplitude of muscle activity necessary to reach a given target (Berardelli et al., 1986; Godaux et al., 1992), causing an insufficient initial burst of agonist muscle activity

(Berardelli et al., 1996a; Hallett and Khoshbin, 1980). The final cardinal motor sign of PD is resting tremor, characterized by involuntary 4-6 Hz tremulous movements of one or more body parts (Jankovic, 2008).

The movements of PD patients are also known to be quite variable (Freeman et al., 1993; Skodda and Schlegel, 2008); however, visual cueing can help reduce this variability (Nieuwboer et al., 2009). PD patients also exhibit postural instability and a slow, shuffling gait (Bloem et al., 2004). In addition to these motor deficits, PD patients experience difficulties multi-tasking (Benecke et al., 1986; Horstink et al., 1990), increased impulsivity (Miyasaki et al., 2007), and autonomic changes [for review see (Jankovic, 2008)].

Subtypes of PD Across PD patients, there is great variability in the time-course, types, and severity of parkinsonian signs (Evarts et al., 1981; Jordan et al., 1992; Meyer, 1982; Nieuwboer et al., 1998; Salamone et al., 1993; Temperli et al., 2003). Specific signs of PD frequently cluster together in specific patients, suggesting that there are symptomatic subtypes of PD. There is no general consensus on how to define specific subtypes of PD; however, the most widely used classification method divides patients into a postural instability/gait difficulty or tremor dominant category (Doder et al., 2003; Zetusky and Jankovic, 1985). Others have attempted to classify PD subtypes based on cardinal motor signs, patterns of cognitive dysfunction, and age of onset (for review see Selikhova et al., 2009). The clustering of specific parkinsonian signs into symptomatic subtypes suggests that separate pathophysiologies may underlie specific subsets of symptoms. Different symptomatic subtypes could be attributable to varying degrees of striatal or extrastriatal DA loss. Teasing apart the specific behavioral effects of DA receptor blockade in specific BG nuclei could help clarify the pathophysiology of specific parkinsonian signs and potentially lead to the development of more individualized therapies.

Here, I will determine how DA loss in the striatum, one specific nucleus of the BG, may contribute to the development of specific parkinsonian signs.

1.2.2 Pathology of Parkinson's disease

Parkinsonian signs are thought to be attributable to striatal DA loss that occurs following the degeneration of the SNpc (Kish et al., 1988). The degeneration of dopaminergic SNpc neurons begins in the lateral tier of the SNpc and proceeds to the medial tier over time. Intracellular inclusions called lewy bodies also develop in the SNpc cells (Forno, 1996), the locus cereleus, the nucleus basalis of meynert, the amyglada, the hippocampus, and the dorsal motor nucleus of the vagus in PD patients as well. The progression of Lewy body pathology through the brain has been used to divide the progression of PD into six stages (Braak et al., 2003). Stage one only involves the motor nucleus of the vagus. The substantia nigra and cortex do not exhibit lewy bodies until stages three and six, respectively. Parkinsonian signs do not typically appear until stage three, when at least 60% of DA neurons in the SNpc are lost, resulting in a nearly total depletion of striatal DA.

Striatal DA loss in PD The earliest and most pronounced DA loss in PD is found in the posterolateral, or motor area of the putamen (Kish et al., 1988). Therefore, DA loss in this region is thought to cause the motor signs of PD. The importance of DA loss in specific striatal subregions presently remains unclear, however. Perhaps DA loss in separate striatal sub-regions contributes to specific signs of PD (Hallett, 1990; Joel and Weiner, 1994). This hypothesis is supported by studies demonstrating that the dopaminergic denervation of the BG motor subcircuit causes parkinsonian motor deficits (Annett et al., 1995; Dunnett and Iversen, 1982; Salamone et al., 1993), while DA depletion in associative and limbic circuits impairs motivation

(Salamone et al., 2003). Here, I will test the hypothesis that different signs can be elicited by DA loss in separate striatal territories.

Support for the idea that striatal DA loss causes parkinsonian signs comes from the observation that DA medications dramatically reduce parkinsonian signs (Perez-Lloret and Rascol, 2010). It remains unclear, however, whether the therapeutic efficacy of these medications is solely attributable to restoring striatal DA levels. Indeed, widespread DA loss is observed in the caudate, pallidum cortex, and thalamus later in the disease (Wichmann and DeLong, 2003) due to the degeneration of dopaminergic SNpc neurons that collateralize to extrastriatal sites (Cossette et al., 1999; Hedreen, 1999) and to degeneration of VTA neurons (Damier et al., 1999; Gibb and Lees, 1991; Javoy-Agid and Agid, 1980; Uhl et al., 1985). This profound extrastriatal DA loss could contribute to the development of specific signs. Thus, DA medications may reduce parkinsonian signs by restoring extrastriatal DA. The therapeutic benefits of restoring striatal DA and extrastriatal DA specifically remain unclear, and should be delineated. I will now discuss how extrastriatal DA loss at specific sites might be implicated in the development of parkinsonian signs.

Cortical DA loss Post-mortem studies in humans report a loss of cortical dopaminergic innervation (Gaspar et al., 1991), and reduced levels of cortical DA (Scatton et al., 1983; Scatton et al., 1982) in PD patients. However, it is unclear whether these findings represent disease progression or a response to long-term DA medication. Furthermore, the behavioral implications of cortical DA loss remain unknown. Sawaguchi and colleagues (Sawaguchi, 2000) report that D1-receptor blockade in the macaque PMd attenuates the directional tuning of PMd cells and impairs reaction and movement times. Thus, cortical DA loss in PD may impair the initiation of movements by reducing the directional tuning and information specificity of cortical cells. In

sum, cortical DA loss occurs in PD and there is evidence that cortical DA loss can alter neuronal activity and affect movement initiation and execution. Therefore, cortical DA loss may contribute to akinetic or bradykinetic signs of PD.

DA loss in the GPe, GPi, STN, and thalamus Like the cortex, the GPe and GPi are innervated by dopaminergic fibers from the VTA and SNpc and receive dopaminergic collaterals from nigrostriatal projections (Cossette et al., 1999; Hedreen, 1999; Nobin and Bjorklund, 1973). The degeneration of these neurons in PD causes considerable DA loss in both pallidal segments (Whone et al., 2003). One post-mortem study reported a staggering 82% and 51% loss of DA in the GPe and GPi, respectively. Some postulate that pallidal DA might be important for functional compensation early in PD following striatal DA loss. Thus, pallidal DA loss may contribute to the worsening of parkinsonian symptoms.

While considerable, the amount of DA loss in the GP is less substantial than the amount of DA loss in the caudate (89% loss) and putamen of the same PD patients (98.4% loss)(Rajput et al., 2008). The widely varying degree of DA loss across the striatum, GPe, and GPi in PD suggests that dopaminergic fibers innervating these nuclei may be distinct and may degenerate independently. This further supports the idea that separate symptomatic subtypes of PD might be attributable to differing degrees of DA loss in the GPe and GPi.

Like the GP, the STN and thalamus suffer substantial DA loss in PD and animal models of parkinsonism (Francois et al., 2000; Freeman et al., 2001; Scatton et al., 1983; Scatton et al., 1982). The behavioral implications of DA loss in the GP, STN, and thalamus remain unclear. However, as previously mentioned, intra-GPe and intra-STN DA receptor blockade induce catalepsy in rodents (Costall et al., 1972; Hauber, 1998). This suggests that DA loss in the GPe and STN could impair motor behavior. The clinical relevance of PD-related DA loss in the

striatum, cortex, GP, STN, and thalamus remain unknown, should be studied in future research, and considered in the development of future PD therapies.

1.2.3 Treatments for Parkinson's disease

First line therapies for PD include DA agonists or levodopa-carbidopa (Perez-Lloret and Rascol). These medications can ameliorate parkinsonian signs by increasing DA levels in the brain. Since PD is progressive, however, these medications become less effective over time. Furthermore, many patients develop debilitating dyskinesias, or excessive movements, in response to therapeutic doses of dopaminergic drugs (Fabbrini et al., 1987).

Deep Brain Stimulation (DBS) is a PD therapy that reduces dyskinesias and parkinsonian signs (Fung et al., 2002). DBS requires neurosurgical placement of a macroelectrode into the motor region of GPi or the STN. The stereotaxic location of an implantation target is typically identified by combined structural imaging and microelectrode recording. Once a target has been identified, a DBS electrode is implanted and connected to a stimulator implanted under the patient's skin. High frequency (>100-Hz) electrical stimulation through the macroelectrode reduces parkinsonian signs rapidly as long as stimulation continues (for a review, see Tomaszewski and Holloway, 2001). Surprisingly, the mechanism whereby DBS ameliorates parkinsonian signs remains unclear.

Gene and transplant therapies for PD are also currently in development (for review, see Obeso et al., 2010). These therapies are based on a simple idea: to restore depleted DA levels in the striatum. Thus far, no gene or DA cell transplant therapy has shown much clinical improvement above placebo (Freed et al., 2001; Goetz et al., 2008; Olanow et al., 2003). This could be due in part to the complexity of the anatomic and physiologic properties of the striatum

mentioned previously. Without a clear understanding of the degree to which DA loss in specific regions of the striatum contributes to parkinsonian signs, it is difficult to identify ideal striatal targets for these therapies. Here, I will determine whether striatal DA receptor blockade in striatal subregions is sufficient to induce specific parkinsonian signs in an effort to validate or exclude potential target locations for intrastriatal therapies.

1.3 PATHOPHYSIOLOGY OF PARKINSON'S DISEASE

The mechanism whereby striatal DA loss causes parkinsonian signs is currently a topic of great debate. According to the classic rate model, DA depletion in the striatum leads to increased activity in the GPi, thereby inhibiting the activity in motor cortical areas and impairing movement (Compare Figures 4 and 1) (Albin et al., 1989; DeLong, 1990; Miller and DeLong, 1987; Wichmann and DeLong, 1996).

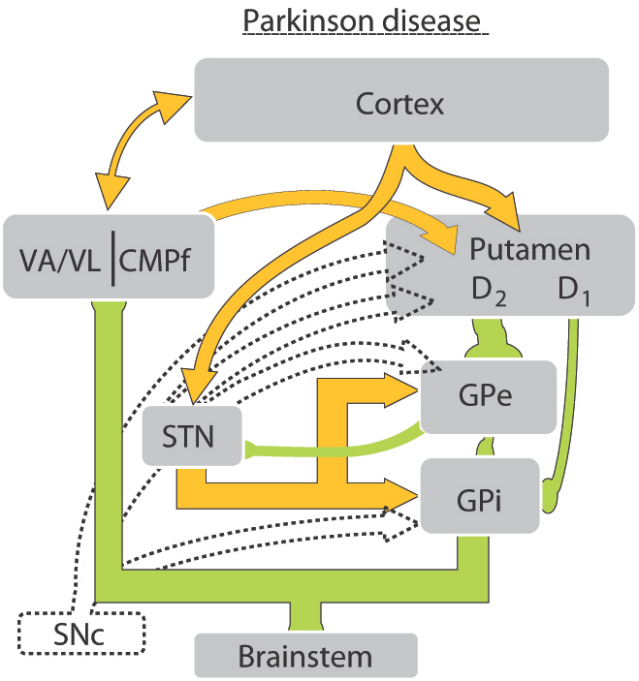

Figure 4: Pathophysiology of Parkinson's disease and the rate model

This figure was reproduced from with permission from the author (Turner, 2009).

Consistent with the rate model, there is evidence that GPi firing rates are elevated in both parkinsonian macaques (Filion and Tremblay, 1991; Miller and DeLong, 1987) and in PD patients (Hutchison et al., 1994; Starr et al., 2005; Sterio et al., 1994). Other studies, however, failed to find changes in the average firing rate of the GPi (Raz et al., 2000) and instead suggest that abnormal activity patterns, such as bursts (Bergman et al., 1994; Boraud et al., 1996; Boraud et al., 1998; Boraud et al., 2000; Filion and Tremblay, 1991; Hutchison et al., 1997), oscillations (Bergman et al., 1994; Filion and Tremblay, 1991; Hutchison et al., 1997; Levy et al., 2002b; Miller and DeLong, 1988; Nini et al., 1995), synchrony (Goldberg et al., 2002; Hurtado et al., 1999; Levy et al., 2000; Nini et al., 1995; Pessiglione et al., 2005; Raz et al., 2000), and reduced selectivity of neuronal activity (Bergman et al., 1998; Filion et al., 1994; Nini et al., 1995) might play a more important role in the pathophysiology of PD. Further support for the rate model is often drawn from the fact that GPi lesions reduce parkinsonian signs (Laitinen et al., 1992).

However, GPi lesions may reduce parkinsonian signs by disrupting the effects of aberrant BG output on cortical activity, rather than by simply suppressing GPi activity. Without more concrete support, the rate model remains theoretical and the pathophysiology of PD remains unknown.

Animal Models of PD Most of what we know about the pathophysiology of PD has come from animal models of the disease. The two most commonly used neurotoxins in animal models of PD include 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). These agents kill dopaminergic neurons in the SNpc, causing both striatal and extrastriatal DA loss (for review, see Emborg, 2007). There is also evidence that 6-OHDA and MPTP cause denervation of dopaminergic neurons that project to the thalamus, GP, STN, and cortex (Debeir et al., 2005; Freeman et al., 2001). Furthermore, MPTP affects the levels of other neurotransmitters, such as norepinephrine and serotonin, in the cortex and cerebellum (Pifl et al., 1991). Neither animal model of PD can therefore rule out the potential role of extrastriatal DA loss, degeneration of SNpc neurons, chronic striatal DA depletion, or changes in other neurotransmitter levels in the development of specific parkinsonian signs. Each of these factors may be involved in the development of specific signs. Therefore, the long-accepted claim that striatal DA loss is the sole cause of parkinsonian signs, and therefore should remain the primary therapeutic target, remains questionable.

Despite our uncertainty about the specific cause of parkinsonian signs, many therapies are currently being developed with the primary goal of restoring striatal DA levels (for review, see Obeso et al., 2010). In order to most effectively design these treatments and tailor them to individual patients' symptomatic profiles, it is critical to understand the degree to which striatal DA loss actually contributes to the development of specific parkinsonian signs. Infusing DA

antagonists into striatal sub-regions represents one approach to answer this question.

In rodents, infusions of DA antagonists into the striatum are known to elicit catalepsy (Amalric and Koob, 1989; Ellenbroek et al., 1985; Hauber et al., 2001; Kaur et al., 1997; Salamone et al., 1998; Yoshida et al., 1994). While rodent studies are certainly informative, behavioral correlates of specific human parkinsonian signs (i.e., bradykinesia vs. akinesia) can be difficult to delineate in these animals. Furthermore, not all rodent studies involving intra-striatal DA receptor blockade report similar findings. In one study, intra-striatal DA receptor blockade attenuated lever pressing for food in a manner resembling extinction (i.e. the attenuation of a behavioral response to a task that no longer provides rewards) without inducing behavioral changes consistent with parkinsonian signs. This provides evidence that striatal DA loss can occur without inducing parkinsonian signs (Beninger et al., 1993).

Experiments on parkinsonian macaques differ from rodent studies in that they can involve more complex cognitive tasks that permit the independent study of specific behavioral changes that more closely mimic human parkinsonian signs. Only one previous study has performed intra-striatal infusions of DA antagonists into macaques (Nakamura and Hikosaka, 2006). These infusions selectively impaired the reward-dependent modulation of saccadic reaction times. Like the work by Beninger and colleagues (1993), this study reported changes in reward-dependent responding without any overt parkinsonian signs. Importantly, however, injections in this study were only performed in the caudate. To my knowledge, no previous study has examined the behavioral effects of intra-putamen DA receptor blockade in macaques. Here, I will determine whether intra-putamen DA receptor blockade induces behavioral changes that are consistent with parkinsonian signs. Potential mechanisms whereby striatal DA loss could induce parkinsonian signs will also be explored.

1.3.1 Abnormal firing properties of the parkinsonian Basal Ganglia

Two overarching hypotheses could explain how striatal DA loss may induce parkinsonian signs: 1) a degradation of a normal function of the BG; or 2) dysfunction of BG-recipient cortical or brainstem regions. These hypotheses are difficult to tease apart, however, without knowing how striatal DA loss alone affects cortical and BG activity. It remains unclear whether striatal DA loss is even sufficient to induce abnormal neuronal activity patterns associated with the parkinsonian state. These abnormal neuronal activity patterns in the parkinsonian BG will now be described in detail, followed by a discussion of the abnormal neuronal activity patterns that have been reported in the parkinsonian cortex.

BG Firing Rates As previously mentioned, striatal DA depletion is thought to decrease GPe activity and increase GPi activity (See Figure 4). There is evidence that GPi firing rates are elevated in both MPTP-treated parkinsonian macaques (Filion and Tremblay, 1991; Miller and DeLong, 1987) and in PD patients (Dogali et al., 1994; Hutchison et al., 1994; Starr et al., 2005). However, it is unclear whether striatal DA loss alone can actually induce these rate changes. Here, I will determine whether striatal DA loss is sufficient to induce rate changes in the BG that are consistent with the rate model.

Bursting in the BG In the normal BG, burst discharges, or sudden increases in firing rate, are relatively rare. In the parkinsonian BG, the prevalence of burst discharges dramatically increases (Bergman et al., 1994; Boraud et al., 1996; Boraud et al., 1998; Boraud et al., 2000; Filion and Tremblay, 1991; Hutchison et al., 1997; Kaneoke and Vitek, 1996; Starr et al., 2005; Wichmann and Soares, 2006). Medical and surgical therapies that reduce PD motor signs do not consistently reduce bursting activity in the BG (Chen et al., 2001; Hahn et al., 2008; Levy et al., 2001; McCairn and Turner, 2009). The mechanistic underpinnings of elevated bursting activity

and its true clinical significance in PD remain unclear. I will determine whether intrastriatal DA receptor blockade can increase bursting activity in the BG, and whether this is related to the severity of specific parkinsonian-like signs.

Oscillatory Activity in the BG In addition to increased burst discharges, oscillatory firing (abnormal rhythmic modulations in firing rate) in the α- (8-13-Hz) and β-(13-30-Hz) frequency ranges is a common characteristic of BG activity in both PD patients and animal models of PD and is thought to play an important role in the pathophysiology of PD (Gatev et al., 2006; Levy et al., 2002bb; Rivlin-Etzion et al., 2006aa; Starr et al., 2005; Weinberger et al., 2006). Treatments that ameliorate PD signs [e.g., dopamine replacement therapy (DRT)] also reduce α- and β-frequency oscillations in spiking (Heimer et al., 2006; Levy et al., 2001) and local field potential (LFP) activity (Brown et al., 2001) in the GPi. Other anti-parkinsonian therapies, such as DBS, also reportedly decrease α- and β-frequency oscillations in the GPi (McCairn and Turner, 2009); however, other studies do not support these findings (Foffani et al., 2006). Therefore, the pathophysiologic role of oscillations in the parkinsonian state remains unclear. Here, I will determine whether intrastriatal DA receptor blockade is sufficient to increase oscillatory activity in the BG and cortex. I will focus particularly on the β-range due to its well-established association with PD.

β-Oscillatory Activity in the BG In the normal brain, β-LFP activity in cortex is suppressed just prior to movement (Kilner et al., 2003). There is evidence that synchronized oscillatory activity in the β-range might suppress movement by disturbing rate coding necessary for voluntary movement (Murthy and Fetz, 1996). Specifically, single unit firing in the M1 is suppressed during periods of 20-40 Hz oscillatory synchrony. Furthermore, firing rate modulations in M1 are more likely to occur as oscillations decrease (Donoghue et al., 1998). It

is also possible that β-activity suppresses movement by interfering with oscillatory activity at other frequencies that are necessary for movement (Brown and Williams, 2005). Finally, β-activity might represent a physiologic feature of the motor system in an idle state (Pfurtscheller et al., 1996) and may suppress movement by simply promoting the existing motor state (Gilbertson et al., 2005).

In general, the β-synchronization of BG nuclei is thought to be akinetic in nature (Brown and Williams, 2005). Stimulating at β-frequencies in the BG (Fogelson et al., 2005; Timmermann et al., 2004) or cortex (Pogosyan et al., 2009) exacerbates bradykinesia. Since β-activity is thought to reflect an akinetic state, it is not surprising that β-activity is more prominent in the BG of parkinsonian patients than in controls (Brown et al., 2001; Levy et al., 2002a; Silberstein et al., 2003). Dopaminergic therapies suppress β-oscillatory LFP activity in PD patients, but therapeutic DBS does not consistently reduce β-activity (Giannicola et al., 2010). There is no additive effect of using both DBS and dopaminergic treatments to suppress β-activity, suggesting that β-oscillations might be more related to DA levels or to specific parkinsonian signs that are more effectively resolved by dopaminergic medications than DBS. Interestingly, the degree of treatment-induced suppression of β-activity in the STN is correlated with the degree of improvement in parkinsonian signs (Kuhn et al., 2008). Taken together, these studies support the idea that β-oscillatory activity is associated with the pathophysiology of PD.

Conversely, there is evidence that oscillations in single cells emerge too late after MPTP administration to contribute to the development of akinesia and bradykinesia in parkinsonian monkeys (Leblois et al., 2007). Therefore, like bursting, the mechanisms and clinical significance of oscillatory firing remain a topic of debate (Degos et al., 2009; Leblois et al.,

2007; Mallet et al., 2008). Ascertaining the mechanism and functional role of β-oscillatory activity in the development and progression of parkinsonian signs will require future research.

The relationship between oscillatory activity and bursting in the BG The oscillations in neuronal firing rate associated with parkinsonism are often described as periodic bursts of neural activity, which has led to the frequent assumption that oscillations and bursts are closely linked, co-occurring phenomena (Rubin and Terman, 2004; Terman et al., 2002). For example, Galvan and Wichmann suggested that oscillations may be caused by rebound bursting within BG loops (Galvan and Wichmann, 2008). In contrast, based on theoretical considerations, Kaneoke and Vitek proposed that oscillations and bursts may represent two distinct processes. Existing evidence that they are separate phenomena includes the following observations: 1) not all oscillatory firing is bursty (Wichmann and Soares, 2006); 2) bursts and oscillations are not affected in a similar manner by pharmacologic and surgical therapies (see above), and 3) bursts and oscillations of neuronal activity in the GPi of human PD patients were found to be independent (Chan et al., 2011). As a result, the specific cause and pathophysiologic relevance of each of these abnormal neuronal activity patterns should be considered separately. Here, I will determine whether striatal DA loss is sufficient to induce bursts or oscillations in neuronal activity.

Increased synchrony in the parkinsonian BG The parkinsonian state is also associated with changes in the integrity of the functionally-segregated cortico-BG-thalamocortical loops mentioned previously. In the normal state, the functional segregation of BG-thalamocortical information circuits has been demonstrated at the single cell level by the following observations: 1) neighboring neurons fire independently from one another (Nini et al., 1995), and exhibit different responses to 2) active (Delong et al., 1984) and passive movements (Delong et al.,

1984; Filion et al., 1988) of particular limbs. The parallel and independent nature of these circuits is thought to allow the simultaneous processing of multiple information circuits through the BG, thalamus, and cortex. In the parkinsonian state, there is evidence that the functional segregation of these circuits is lost (Bergman et al., 1998). The activity of cells in the BG, BG-recipient thalamus, and M1 becomes less selective and more synchronous (Filion et al., 1988; Goldberg et al., 2002; Pessiglione et al., 2005). It has been suggested that this loss of functional segregation decreases the brain's capacity to process multiple streams of information in parallel (Hammond et al., 2007), which could slow movement initiation and execution. The cause of functional de-segregation in the parkinsonian state presently remains unclear.

The degree of functional segregation is most accurately quantified by determining the degree to which neuronal activity is selective and whether the activity of two neurons occurs independently or synchronously. Reduced response selectivity and increased synchronous activity would suggest a loss of functional segregation. Since these analyses were not possible with the neuronal data collected here, LFP signals were examined instead.

LFPs represent low-frequency signals that are thought to reflect synchronized synaptic activity generated by large populations of neurons (Haberly and Shepherd, 1973; Mitzdorf, 1985; Rebert, 1973). The degree to which LFP activity actually represents the spiking of nearby neurons is unclear and it likely differs between brain regions and neuronal types. There is evidence, however, that changes in LFP fluctuations are related to changes in neuronal activity, at least in the striatum and STN (Courtemanche et al., 2003; Goldberg et al., 2004; Goto and O'Donnell, 2001; Levy et al., 2002a). One way to quantify synchronous activity using neuronal activity and LFP signals is by determining the number of cells with spiking activity that are time-locked, or synchronized with, fluctuations in LFPs. These spike-triggered LFPs can also be used

to determine whether or not synchronous activity is oscillatory in nature. Thus, LFP signals are useful in providing information about the degree to which neuronal activity is synchronized and how it is synchronized (e.g., rhythmic at a specific frequency or arrhythmic). While increased synchrony is known to occur in the parkinsonian BG, the mechanism and clinical relevance of this increase remains unclear. Here, we will test whether striatal DA loss is sufficient to enhance synchronous activity in the BG.

In summary, the parkinsonian BG is characterized by alterations in firing rate, increased burstiness, increased oscillatory activity, and increased synchronous activity. It remains unknown whether these changes in neuronal firing are attributable to striatal DA loss. Furthermore, the clinical relevance of these changes in neuronal activity actually remains unclear. These abnormalities may induce parkinsonian signs by disrupting cortical activity. Indeed, there is a great deal of evidence that cortical activity is altered in the parkinsonian state.

1.3.2 Cortical activity in the parkinsonian state

Only a handful of studies have evaluated cortical changes in single cell activity patterns in the parkinsonian state. While few in number, all of these studies suggest that PD-related changes in cortical activity patterns may contribute to the pathophysiology of parkinsonian motor signs (Doudet et al., 1990; Escola et al., 2003; Goldberg et al., 2002; Pasquereau and Turner, 2010).

M1 The activity of M1 cells is more synchronous, more bursty, and less specific to passive limb movements in MPTP-treated macaques (Goldberg et al., 2002). Directional tuning and peri-movement discharge are also reduced in parkinsonian monkeys (Turner, 2007). While many studies found no evidence for reduced firing rates in the M1 of parkinsonian animals (Doudet et al., 1990; Goldberg et al., 2002; Parr-Brownlie et al., 2007), others reported that M1

firing rates are indeed suppressed in the parkinsonian state (Parr-Brownlie and Hyland, 2005; Pasquereau and Turner, 2010). In sum, alterations in M1 activity and firing patterns have been reported in the parkinsonian state and may represent the pathophysiologic substrate of impaired movement execution in PD.

Pre-motor activity The activity in other movement-related areas of the cortex is altered in the parkinsonian state as well. Cells in the SMA exhibit impaired neuronal responses to visual cues and irregular activity patterns in MPTP-treated macaques (Escola et al., 2003). There are many other reports of task-related changes in the activity of several pre-motor cortical areas in PD patients as well (Carbon et al., 2007; Haslinger et al., 2001; Jahanshahi et al., 1995; Playford et al., 1992; Samuel et al., 1997; Turner et al., 2003). Imaging studies in humans also report abnormal hyperactivation of the PMd (Carbon et al., 2007; Catalan et al., 1999; Cerasa et al., 2006; Haslinger et al., 2001; Samuel et al., 1997) that may be related to the impaired initiation of movements in PD patients (Carbon et al., 2007). There are conflicting reports as to whether pre-motor and motor activity is increased or decreased in the parkinsonian state. However, all studies concur that the parkinsonian state is associated with changes in pre-motor and motor cortex activity. The cause of PD-related changes in premotor and motor cortex activity remains unclear, but there is evidence that striatal DA loss could be involved.

Influence of striatal DA levels on cortical activity Intrastriatal infusions of D1 receptor agonists were shown to enhance motor cortex c-Fos expression in rats (Blandini et al., 2002; Blandini et al., 2003), while increases in cortical c-Fos expression induced by systemically administered DA agonists (LaHoste et al., 1996) were blocked by intrastriatal injections of D1 antagonists (Steiner and Kitai, 2000). While c-Fos only represents an indirect measure of neuronal activity, these studies support the idea that striatal DA levels can affect cortical activity.

Intrastriatal D1 blockade was also found to impede behavioral recovery following M1 damage in rats (Davis et al., 2007). Together, these studies support the idea that changes in striatal DA levels could alter cortical activity. However, the mechanism of this interaction remains unclear. A few possibilities will now be addressed.

Abnormal activity patterns in the BG that develop in response to striatal DA loss could be directly transmitted to cortex via reverberations through BG-thalamocortical loops. Should the activity of large cortical cell populations become entrained to nonspecific oscillatory activity generated by the BG, the selective activation of distinct cortical neuronal populations may be impaired. Indeed, combined EEG and LFP studies in PD patients demonstrate that the BG adopts low frequency oscillations from cortex in the DA-depleted state (Williams et al., 2002). Pharmacologically restoring DA levels allows the BG to send normal, high frequency oscillations to the SMA. This supports the idea that impaired processing within the BG may alter cortical activity. It is unclear whether this impaired processing in the BG was solely attributable to striatal DA loss, however, as extrastriatal DA levels were likely restored by the DA medications as well.

Striatal DA could also affect cortical activity directly via presynaptic receptors (Garcia-Munoz et al., 1991) or indirectly by affecting the activity of SNpc neurons which then modulate cortical DA levels. Regardless of the mechanism, there is support for the idea that striatal DA loss disrupts cortical activity. Here, I will determine whether striatal DA receptor blockade is sufficient to alter cortical activity in a manner consistent with the parkinsonian state. I will also test for similar changes in the BG. Finally, changes in neuronal activity in both the cortex and BG will also be related to the severity of parkinsonian signs following striatal DA loss.

1.4 SUMMARY AND AIMS

PD patients exhibit a multi-faceted symptomatic profile, primarily characterized by rigidity, tremor, bradykinesia (slowness of movement), and akinesia (difficulty initiating movement). Many PD patients suffer from cognitive, autonomic, and postural deficits as well. Despite such a diverse symptomatic profile, PD has long been attributed to one simple cause: loss of DA in the striatum. The striatum, or input structure to the BG, is organized into functional territories that are thought to play separate roles in cognitive and/or motor functioning. Possibly, DA loss in separate striatal functional territories contributes to different parkinsonian signs. Presently, however, the pathophysiology of specific parkinsonian signs is becoming less clear, with emerging evidence suggesting that extrastriatal DA loss and changes in the other neurotransmitter levels may also be involved. Therefore, it is important to test the long-accepted claim that striatal DA loss causes the cardinal signs of PD. Specifically, is the blockade of striatal DA receptors sufficient to elicit specific parkinsonian signs?

The under-activation of striatal D1 and D2 receptors has long been thought to impair movement in PD by tonically increasing the activity in the GPi, thereby indirectly suppressing activity in the M1. More recent evidence, however, demonstrates that the activity of M1 cells becomes more synchronous, bursty, and less directionally-tuned in the parkinsonian state, *without* any changes in overall firing rate. Similarly, the parkinsonian BG is characterized by changes in single cell activity patterns such as bursts, oscillations, and synchrony. It remains unclear whether these changes are attributable to striatal DA loss. Furthermore, the pathophysiologic relevance of abnormal firing patterns in the cortex and BG remains unknown. There is mounting evidence, however, that abnormal firing patterns may play a more important role in the pathophysiology of PD than changes in firing rates.

Here, we will determine whether focal, temporary DA receptor blockade in striatal sub-regions is sufficient to 1) elicit specific parkinsonian signs; and 2) induce abnormal neuronal activity patterns in the BG and cortex that are associated with the parkinsonian state. We will also relate the degree of change in neuronal activity with the severity of parkinsonian signs. In this way, we will test the fundamental hypothesis that striatal DA loss is sufficient to induce the behavioral and neural correlates of PD, while exploring whether specific abnormal neuronal activity pattern changes are related to the severity of separate parkinsonian signs.

2.0 BEHAVIORAL EFFECTS OF INTRASTRIATAL DA RECEPTOR BLOCKADE

Parkinson's disease (PD) is often attributed to one simple deficit: a loss of dopamine (DA) in the striatum due to the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (for review, see Forno, 1996; Kish et al., 1988). Overwhelming evidence now indicates that PD is also associated with a wide variety of other pathologic defects. For example, the nigrostriatal dopaminergic neurons that degenerate in PD collateralize to other sites (Cossette et al., 1999; Hedreen, 1999). As a result, DA loss in the parkinsonian brain is not restricted to the striatum. The subthalamic nucleus, the thalamus, the globus pallidus (GP), and cortex all suffer substantial DA loss in both PD and animal models of parkinsonism (Francois et al., 2000; Freeman et al., 2001; Scatton et al., 1983; Scatton et al., 1982).

Like the dopaminergic system, the serotonergic and noradrenergic systems also exhibit neuronal degeneration in PD (Scatton et al., 1983). Substantial non-dopaminergic cell loss also occurs in the brainstem [i.e., the pedunculopontine nucleus (Bohnen and Albin, 2010; Zweig et al., 1989) and the motor nucleus of the vagus (Braak et al., 2003)] and the intralaminar thalamic nuclei (Henderson et al., 2000).

Considering this diverse and widespread pathology in PD, it has become increasingly unclear which pathologic defects contribute to the genesis of parkinsonian signs. While some PD-related CNS changes may play direct roles in the development of parkinsonian signs, others may simply share a common pathogenesis without directly contributing to parkinsonian signs

(Forno, 1996). The minimal pathologic defect necessary to induce parkinsonian signs remains unknown. Here, we sought to establish whether striatal DA loss is sufficient to induce specific signs of parkinsonism.

Current neurotoxin models of PD cannot be used to answer this question for a variety of reasons. Neurotoxins, such as 6-OHDA and MPTP, kill neurons in the SNpc, causing loss of dopamine in the striatum and at extra-striatal sites (Debeir et al., 2005; reviewed by Emborg, 2007; Freeman et al., 2001; Pifl et al., 1991). Even infusions directly into the striatum result in a loss of dopaminergic terminals at multiple extra-striatal sites (Debeir et al., 2005; Freeman et al., 2001) due, presumably, to retrograde degeneration of somata in the SNpc (Oiwa et al., 2003; Sauer and Oertel, 1994) and of the extensive multi-nuclear arborizations of those cells (Fallon, 1981; Gaspar et al., 1992; Lindvall and Bjorklund, 1979; Prensa and Parent, 2001; Takada and Hattori, 1986). Neurotoxin models, therefore, cannot rule out the potential roles of extra-striatal DA loss, degeneration of SNpc neurons, chronic striatal DA depletion, or changes in other neurotransmitter levels in the development of parkinsonian signs. The growing recognition that PD is associated with a variety of pathologies encourages further consideration of the possibility that different signs arise from different pathologic substrates.

The primary signs of PD include akinesia, bradykinesia, rigidity and tremor. Importantly, the time-course and severity of sub-groups of parkinsonian signs vary independently between patients (Evarts et al., 1981; Jordan et al., 1992; Meyer, 1982; Nieuwboer et al., 1998; Selikhova et al., 2009; Temperli et al., 2003; Zetusky and Jankovic, 1985). It is therefore possible that different parkinsonian signs have distinct pathophysiologic substrates. To determine whether striatal DA loss is sufficient to cause specific parkinsonian signs, we

manipulated striatal DA transmission using local infusions of a D1- and D2-receptor antagonist in macaques while quantifying aspects of motor performance reflective of parkinsonian signs.

Infusion of DA receptor antagonists into the striatum is known to elicit catalepsy in rodents, a behavioral state thought to reflect signs of rigidity and akinesia (Amalric and Koob, 1989; Ellenbroek et al., 1985; Hauber et al., 2001; Kaur et al., 1997; Salamone et al., 1998; Yoshida et al., 1994). Surprisingly, only one previous study has examined the behavioral effects of striatal DA blockade in non-human primates (Nakamura and Hikosaka, 2006). In that study, small intra-caudate infusions of D1- or D2-specific antagonists disrupted the normal variation of oculomotor reaction times with the size of a reward (Nakamura and Hikosaka, 2006), but did not elicit signs of parkinsonism. By infusing large volumes of a D1/D2-receptor antagonist at various sites in the putamen while animals performed a bimanual reaching task, we were able to test whether acute blockade of striatal DA transmission is sufficient to cause specific parkinsonian signs, and whether suppressing DA activity in distinct striatal sub-regions elicits separate parkinsonian signs.

2.1 METHODS

2.1.1 Animals and Task

Two monkeys (*Macaca mulatta*; D, male ~7.5 kg; E, female ~6 kg) participated in the study. All aspects of animal care were in accord with the ‘‘Guide for the Care and Use of Laboratory Animals’’ (National Academy Press, 1996), and all procedures were approved by the institutional animal care and use committee. An animal was seated in a primate chair facing a vertically-mounted response panel ~31 cm in front of the animal's sternum. The panel contained

two identical bi-colored LEDs, approximately aligned with the animals' left and right shoulders. Proximity sensors (Takex, GS20N) were mounted above each LED to detect hand contact with the colored LEDs. Two metal rods (“homekeys”, also with the same type of proximity sensors) were mounted on the left and right sides of the primate chair ~7 cm lateral to the animal's body. The vertical distance from the animals' shoulder to the homekeys was ~26 cm. The linear distance between each homekey and the ipsilateral and contralateral panel-mounted LED was ~34 and ~40 cm, respectively.

The bimanual reaching task required the animal to reach to the lit LED target with the left or right hand to obtain a food reward. Which hand to reach with (left or right) was signaled by the color of the LED target (red or yellow, respectively; See Figure 5).

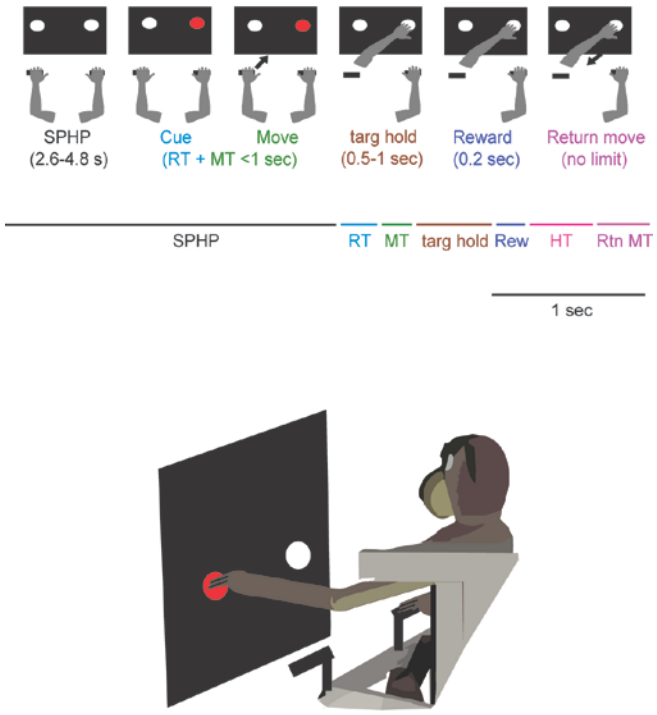

Figure 5: Task schematic

This figure represents a schematic of the reaching task. Each trial began with a mandatory start position hold period (SPHP) in which the animal was required to hold the two homekeys for a variable period of time. The cue was then presented and the animal was required to move to the correct cue with the correct hand and hold the hand at the target for a variable period of time to receive a reward. After the reward, the animal could initiate a return movement to the homekey whenever the animal wanted. There were no cues, time limits, or rewards for this return movement. Each segment of one example trial is also depicted as a straight line, color-coded by segment and drawn to a length that represents the duration of that segment.

During an initial start position hold period (SPHP 1-4 second duration, randomized trial-to-trial following a uniform distribution) left and right hands were required to remain stationary

on their respective homekeys. At the end of the SPHP, one of the target LEDs was illuminated [four trial types (left or right target × red or yellow LED) selected at random]. The animal was required to lift the appropriate hand and touch the correct target in $\leq$ 1 second. The animal's hand was required to remain at the correct target for 0.5-1 second before a drop of liquid food reward was delivered via a sipper tube. Return of the hand to the homekey could be initiated and executed at will, with no time requirements. On 3/26 injection days, the animal performed the task with only the arm contralateral to the infusion site while the ipsilateral arm was loosely restrained in the padded splint. This was done while one of the animals was being trained to accept external EMG leads on the contralateral arm.

2.1.2 Structural MRI

Prior to implantation surgery, MRI images were obtained using a Magnetom Allegra 3 Tesla MR scanner (Siemens Medical Solutions USA, Malvern, PA). The animal was anesthetized with Isoflurane and placed into a MR-compatible stereotactic holder (Crist Instrument Co., Hagerstown, MD) built into an MR transmit/receive coil (Nova Medical Inc., Wilmington, MA). The birdcage-style coil had an inside diameter of 16 cm, and operated in quadrature mode. Three-dimensional T1 images of the whole head were acquired using a MPRAGE sequence with 0.6 mm isotropic voxels. Four successive scans were averaged to improve the signal-to-noise ratio. The resulting images were used for stereotaxic targeting in the subsequent surgery and for later reconstruction of injection locations (see Figure 6).

2.1.3 Surgical Procedure

Many of these methods have been described previously (Desmurget and Turner, 2008). Animals were prepared surgically using aseptic technique under isoflurane anesthesia. Two cylindrical titanium recording chambers (18 mm ID) were affixed to the skull over craniotomies at stereotaxic coordinates to allow transdural access to the right and left putamen and globus pallidus (GP) from a coronal approach. For a detailed description of surgical techniques, see (Turner and Anderson, 1997). The chamber was fixed to the skull with bone screws and dental acrylic. Bolts embedded in the acrylic allowed fixation of the head. After the animal recovered from the implantation surgery, the boundaries of cortex, putamen, and the GP were located with respect to chamber coordinates using standard microelectrode mapping techniques (Turner and DeLong, 2000). The motor putamen was defined by areas that evoked reliable muscle contractions in the leg, arm, or face in response to microstimulation (300-400 Hz, 40-60 μA, 10 pulses, 200-300 μsec pulse duration). The resulting 3-dimensional information regarding the locations of nuclear boundaries was overlaid onto each monkey's MRI using Cicerone, a purpose-designed neurophysiologic mapping software (Miocinovic et al., 2007). Microelectrode mapping continued until putamenal and GP borders could be localized with a high degree of confidence (Figure. 6A).

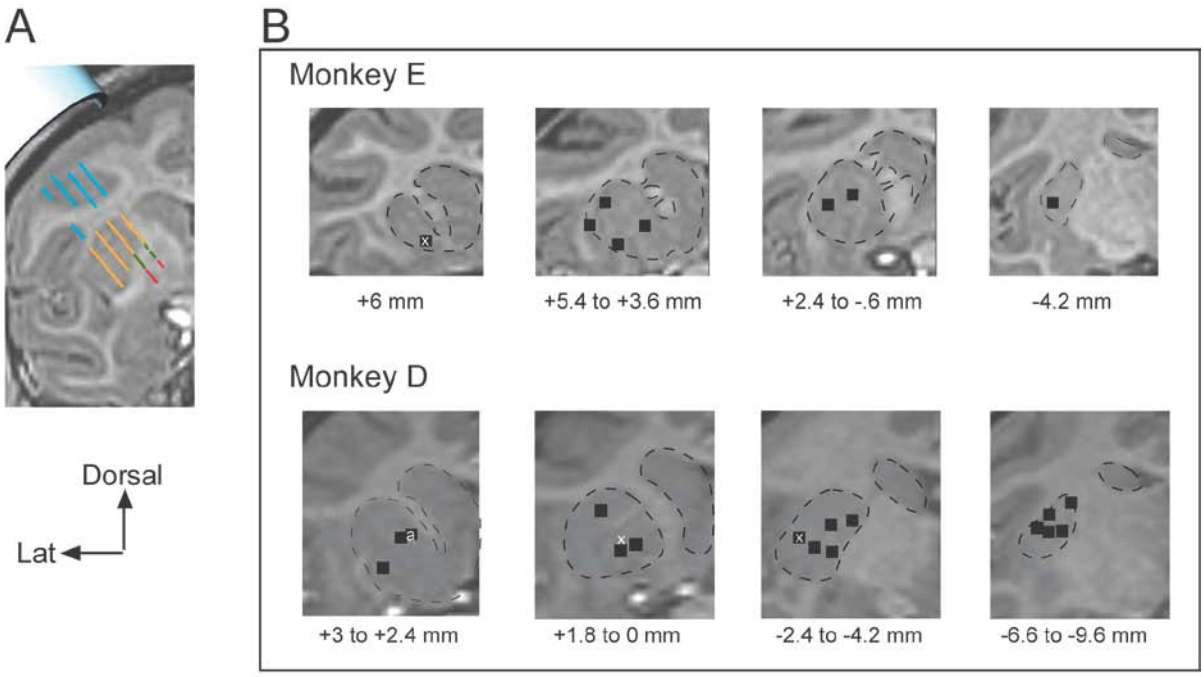

Figure 6: Injection sites

A) Depiction of how neurophysiologic information from recording tracks was merged directly onto the monkey's MRI. Segments of the recording tracks were color-coded based on characteristic neurophysiologic activity known to be associated with distinct brain regions (*blue* = cortex, *orange* = striatum, *green* = GPe, *red* = GPi) and merged onto the monkey's MRI. As seen in one exemplar coronal plane (A), most recordings lined up very well with the animal's MRI. B) For monkey E and monkey D, each injection site is marked with a *black square* (cis-flu) or *white x* (saline). Injection sites are collapsed across coronal slices (< 3 mm spread per section). The distance range below each figure represents the AP planes collapsed into that figure (as mm anterior (+) or posterior (-) to the anterior commissure). For monkey D, the injection sites across both hemispheres were collapsed onto one hemisphere.

Additional microelectrode mapping tracks performed on days following an injection yielded nearly identical locations for the nuclear boundaries, reinforcing the conclusion that this

system allowed reliable targeting of sites within the putamen. The animals used in this study are still alive.

In monkey D, surface EMG was collected from biceps and triceps muscles using gold cup electrodes (Grass, Inc.) and electrode gel attached to the upper arm by a custom-built adjustable strap. These electrodes were removed after each experimental session. In monkey E, pairs of teflon-insulated multi-stranded stainless steel wires were implanted chronically into four muscles of the arm (biceps, triceps, latissimus dorsi, and deltoid muscles) in a separate surgery. The wires were led subcutaneously to a connector fixed to the skull implant. Accurate placement of the wires was verified post-surgically by: 1) determining that each electrode pair provided independent EMG-like signals (thereby ruling out cross talk); and 2) observing palpable contraction of the appropriate muscle when electrical stimulation was applied to each electrode. Following both surgeries, animals were given prophylactic antibiotics and analgesic medication. After recovery from surgery, animals resumed behavioral testing.

2.1.4 Microinjections

Injection Apparatus An evenly-distributed set of putamenal injection sites was selected using microelectrode mapping results, each monkey's MRI, and Cicerone software system (Miocinovic et al., 2007)(Figure 6A). The custom-built microinjection cannula consisted of a piece of fused silica tubing 140 cm in length (99 um ID, 196 um OD, Polymicro Technologies, L.L.C.) glued to a leur-lock fitting, allowing attachment of a 25 µl Hamilton syringe for drug delivery. The end of the fused silica to be lowered into the brain was glued inside of a nested series of stainless steel hypodermic tubing, leaving 3-5 cm of bare silica tubing exposed at the end. Using this design, only silica tubing entered the brain. The tip of the injection cannula was lowered into the

brain through a dura-piercing guide tube held in an mm-resolution grid mounted in a microdrive (MT, Alpha Omega Co. USA). The Hamilton syringe was placed in a motorized syringe pump (Hamilton Co.) which controlled the rate and volume of solution infused.

Injection Protocol Infusions of either cis-flupenthixol (cis-flu; D1 and D2 antagonist, Sigma-Aldrich) or 0.9% sterile saline were performed using convection-enhanced delivery, a safe and effective way to deliver large volumes of solute into the primate brain at a slow constant rate (Bankiewicz et al., 2000; Krauze et al., 2005; Saito et al., 2004). On the day of infusion, cis-flu was dissolved in 0.9% sterile saline (5-10 µg/µl) and filter-sterilized (0.22 micron Milipore filter) before infusion. After filling the injection cannula with cis-flu and attaching it to a Hamilton syringe loaded with cis-flu, the Hamilton syringe was placed into a motorized pump. One-half microliter of sterile saline was then drawn into the tip of the cannula so that any leakage during cannula placement would contain only saline. The tip of the injection cannula was then lowered manually into the brain through a dura-piercing guide tube mounted on a microdrive. Once the cannula was at the appropriate depth, behavioral data from the task was collected before (15-20 min), during (10.5 min), and after each injection. The monkey was allowed to work until it was satiated (total duration of data collection ~90-120 min). The rate (1 µL/min) and volume (10.5 µL) of the infusion was controlled by the syringe pump. The infusion cannula was left in place at the injection site throughout the post-injection recording period.

On saline injection days, 10.5 µl of sterile saline was infused using the same apparatus and identical methods. We infused a large volume of solute in order to maximize the area of tissue affected by drug. The injection was expected to affect a sphere of tissue around the injection site ~4.6 mm in diameter (Vogelbaum, 2005). During 17/26 cis-flu and 2/3 saline injections, 3-4 microelectrodes were also lowered into the cortex, GPe, and GPi through the

same microdrive and chamber to collect neuronal data. Neuronal data collected during these experiments are discussed later (See *Section 3.0*). At the termination of each experiment, the injection cannula and microdrive were removed. Injections were performed, at minimum, 7 days apart. In an effort to minimize backflow attributable to recent damage (Alexander et al., 2010), injection locations were alternated between anterior and posterior regions of the striatum thereby maximizing the distance between two consecutive injections.

Data Collection Behavioral events were stored with 40 μsec accuracy. EMG signals were amplified (×10k), band-pass filtered 100-5000 Hz, sampled at 6104 Hz, rectified, then low-pass filtered at 500 Hz and saved at 1017 Hz. All of the data were collected using a real-time processor (RZ2, Tucker-Davis Technologies). A separate video capture setup (Nuvico EV-4000) recorded the animal's general behavior and movement while performing the task.

2.1.5 Behavioral Analyses

Measures of task performance reflective of akinesia included reaction times (RTs; the time interval between illumination of a target LED and lifting of the appropriate hand from the homekey) and hold times (HTs; the time interval during which the animal's hand remained at the target following reward delivery and before the animal initiated a return movement to the homekey). It is important to differentiate between RTs and HTs, as the former refers to the time interval to initiate an externally-cued and rewarded movement to a visible target location, while the latter refers to the time interval to self-initiate a movement without the benefit of external sensory cues, promised rewards, or a visible target location. Movement times (MTs; the duration of reach movements from the homekey to the appropriate target) were used to quantify the

degree of bradykinesia. The durations of return movements (moving the hand from the target to the homekey) were highly variable, even on non-injection days, and thus were not analyzed.

Significant effects of injections were identified using chi-squared analyses. Due to the long period of time required to inject the total volume of solution (10.5 min), the first 315 seconds of the injection period was considered part of the "pre-injection" period. The beginning of the "post-injection" period included the last 315 seconds of the injection period. A chi-squared analysis was used to compare the number of trials pre- versus post-injection in which a behavioral measure deviated $\geq$ 3.5 standard deviations from the pre-injection mean. A chi-squared analysis was performed on trial-by-trial measures of task performance (RT, HT, MT) separately for each arm (contralateral and ipsilateral to the injection site) and each injection. Behavioral effects were tested for significance using one-tailed chi-squared tests ($p < 0.05$).

The chi-squared analyses were designed to detect injection-induced changes in behavior that may have been expressed variably from trial-to-trial. To supplement that analysis, we also measured the mean magnitude (i.e., severity) of cis-flu induced changes in behavior. A sliding window of 20 consecutive trials of the same trial type (~ 80 trials total) was stepped trial-by-trial through the post-injection data. The within-window mean of a given behavioral measure was normalized by dividing by that measure's pre-injection mean (i.e., 0% indicated no change from pre-injection). The maximum percent change across all positions of the sliding window was taken to represent the magnitude of an injection-related change in behavior. This analysis was performed separately for each of the 4 trial types (left-arm left-target, left-arm right-target, right-arm right-target, right-arm left-target).

Finally, the magnitudes of injection-related changes in RT, MT, and HT for each arm were correlated with each other across injections using Spearman correlation analyses to test for co-variation in the magnitude of different behavioral effects.

2.2 RESULTS

A total of 26 cis-flu injections were performed throughout the putamen in three hemispheres across two monkeys (18 injections across two hemispheres in monkey D, 8 injections in one hemisphere in monkey E). Three saline injections into the putamen were also performed, one in each hemisphere (See Table 1).

Table 1: Summary of injections

Animal	Inj No.	Task	Recording	AC plane	drug	Vol. (µL)	Conc. (µg/µl)	Hemisphere	Significant Effect	Magnitude (% Δ)
D	1	bimanual	no	-3.8*	cis-flu	10.5	5	R	LRT,LHT	28, 52
D	2	bimanual	no	-5.8	cis-flu	10.5	10	R	LRT, LMT, LHT	19, 13, 25
D	3	bimanual	no	-3.8	saline	10.5	10	R	No effect	-
D	4	bimanual	no	-3.8*	cis-flu	10.5	10	R	No effect	-
D	5	unimanual	no	2.7†	cis-flu	10.5	10	R	LRT,LMT, LHT	20, 24, 313
D	6	unimanual	no	-1.8	cis-flu	10.5	10	R	LHT	184
D	7	bimanual	no	2.7	cis-flu	10.5	10	R	No effect	-
D	8	bimanual	no	-1.8	cis-flu	10.5	10	R	No effect	-
D	9	unimanual	no	2.7†	cis-flu	10.5	10	R	LRT	25
D	10	bimanual	yes	3.3	cis-flu	10.5	10	R	LRT,LMT	27, 16
D	11	bimanual	no	-8.8	cis-flu	10.5	10	R	No effect	-
D	12	bimanual	yes	2	cis-flu	10.5	10	L	LRT	31
D	13	bimanual	yes	-9.1	cis-flu	10.5	10	L	RMT	9
D	14	bimanual	yes	-6.1	cis-flu	10.5	10	L	RRT	14
D	15	bimanual	yes	2	cis-flu	10.5	10	L	LRT	71
D	16	bimanual	yes	-2.1	cis-flu	10.5	10	L	No effect	-
D	17	bimanual	yes	-2.1	cis-flu	10.5	10	L	RRT,LRT,LMT, LHT	37, 20, 13, 31
D	18	bimanual	yes	-6.1	cis-flu	10.5	10	L	No effect	-
D	19	bimanual	yes	3	cis-flu	10.5	10	L	No effect	-
D	20	bimanual	yes	1	saline	10.5	10	L	No effect	-
E	21	bimanual	yes	6.2	cis-flu	10.5	10	R	No effect	-
E	22	bimanual	yes	4.2	cis-flu	10.5	10	R	No effect	-
E	23	bimanual	yes	-0.2	cis-flu	10.5	10	R	LHT	37
E	24	bimanual	yes	7.2	cis-flu	10.5	10	R	No effect	-
E	25	bimanual	yes	3.2	cis-flu	10.5	10	R	LHT,RHT	33, 26
E	26	bimanual	yes	7.2	saline	10.5	10	R	LMT	14
E	27	bimanual	yes	4.2	cis-flu	10.5	10	R	LHT	21
E	28	bimanual	yes	-4.2	cis-flu	10.5	10	R	LMT	10
E	29	bimanual	yes	6.2	cis-flu	10.5	10	R	No effect	-

Each injection is listed in the temporal order in which it was performed, with one row per injection. *Task* indicates whether the animal was performing the task uni-manually (contralateral to the infusion) or bi-manually. *Recording* indicates whether or not neuronal activity was simultaneously recorded from cortex, GPe, and/or GPi during the injection. *AC plane* refers to the distance from the injection site (in mm) anterior (+) or posterior (-) to the anterior commissure. *Drug* indicates which solution was infused (cis-flu or saline). *Volume* and *Concentration* indicate the volume and concentration of infusate. *Hemisphere* indicates the hemisphere in which the injection was performed. *Significant effects* are identified by type (LRT = left arm reaction time, LMT = left arm movement time, LHT = left arm hold time, RRT = right reaction time, RMT = right movement time, RHT = right hold time). *Magnitude* indicates the size of each significant effect, respectively (as a percent increase post-injection relative to pre-injection). * and ŧ superscripts denote repeat injections in the same location.

Results from microelectrode mapping overlaid onto the monkeys' MRI were used to determine the location of injections (See Figure 6).

2.2.1 Pre-injection task performance

Both animals performed the bimanual reaching task accurately and reliably each day prior to injections (97 +/- 1% correct and 98 +/- 1% correct; means +/ SEM for monkeys D and E, respectively, across pre-injection data from 26 injections). Mean HTs, during which an animal's hand remained stationary at a peripheral target before initiating a return-to-home movement, were relatively brief and consistent (503 +/- 33 and 527 +/- 20 msec) suggesting that the animals initiated these non-rewarded movements efficiently in order to move to the next rewarded trial. Mean RTs (320 +/- 7 and 343 +/- 5 msec) and MTs (204 +/- 4 and 207 +/- 3 msec) were within ranges that are common for choice reaction time tasks such as this one (Gardinier et al., 2006). On non-injection days, the animals performed an average of 854 ± 24 trials and 1169 ± 60 trials

per day over the course of approximately 1-2 hours per day. Day-to-day variability in the number of trials and the duration of task performance arose due to 1) gradual improvements in task performance over the several months these experiments required; and 2) daily variations in an animal's satiety.

2.2.2 General effects of intrastriatal cis-flu injections

Following intra-striatal injections of cis-flu, animals continued to perform the behavioral task and displayed no gross abnormalities in behavioral state [e.g., no signs of catalepsy, agitation, or intrusion of grossly interfering movements or behaviors (Worbe et al., 2009)]. However, marked changes in task performance were often noted. Figure 7 depicts results from one cis-flu injection (injection site "a" in Figure 6B) that induced significant increases in HT, RT, and MT.

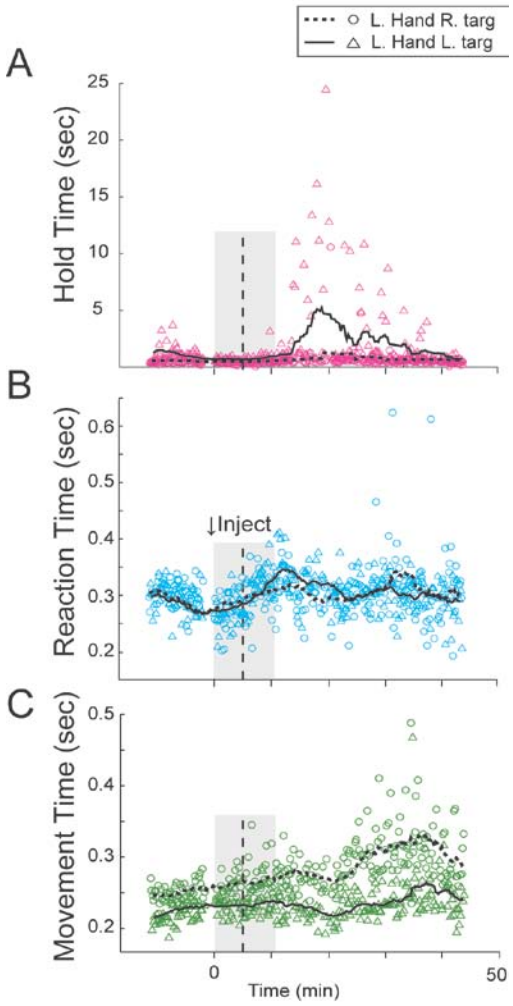

Figure 7: Example cis-flu infusion

This figure depicts exemplar data from one cis-flu injection day. Each data point represents a HT (*pink*), RT (*blue*), or MT (*green*), for separate trials. In this example, the monkey was performing the task with the left arm only (contralateral to the infusion site). The hand and target used for each trial is denoted by the shape of the data point (*circles* indicate the target contralateral to the infusion, while *triangles* denote the target ipsilateral to the infusion). The *shaded box* marks the period during which cis-flu was injected. The *vertical black dotted line* represents the delineation between "pre-" and "post-injection" periods used for all behavioral analyses. The moving averages (20 trials of each trial type) for contralateral and ipsilateral trials are depicted as *dotted* and *straight* lines overlaying the data points, respectively. A) The monkey's HTs were by far the most affected movement parameter. After the infusion, the monkey held its hand at the target for

up to 25 seconds before initiating a return movement. B) The monkey's RTs also increased after the injection, but to a lesser degree. C) The monkey's MTs increased substantially following this cis-flu injection.

Soon after completion of the injection (~5 minutes), the animal exhibited markedly prolonged HTs ($p < 0.05$; $\chi^2 = 9.7$), particularly for movements to the target ipsilateral to the injected hemisphere (454% and 173% increase for ipsi- and contra-lateral trials respectively; See Figure 7A). During each prolonged HT event, the animal's arm appeared to be "frozen" in an elevated and extended posture at the peripheral target. The animal continued to perform normal chewing and licking movements during these freezing events, so it is unlikely that they reflected a general loss of consciousness or awareness. In this example, the prolongation of HTs peaked ~20 minutes following injection onset, was no longer evident ~37 minutes after injection onset, and only appeared in a subset of trials.

The animal's RTs and MTs began to slow as well starting ~20 minutes post injection onset ($p < 0.05$; $\chi^2 = 4.4$; See Figure 7B; $p < 0.0001$; $\chi^2 = 27.7$; See Figure 7C, respectively). The maximum changes in RTs were 18% and 21% increases for reaches to the contralateral and ipsilateral targets, respectively. At the peak of the MT effect, MTs were lengthened by 32% and 17% for reaches to contra- and ipsi-lateral targets, respectively. Note that the magnitude of increases in RT and MT was later and of smaller magnitude than the increases in HTs. Also note that the animal was performing the task with just the arm contralateral to the infusion site during this infusion.

Electromyographic (EMG) activity collected during the same experiment suggested that the prolongation of HTs was not attributable to exaggerated co-contraction of antagonist muscle pairs or a simple loss of muscle tone (See Figure 8A).

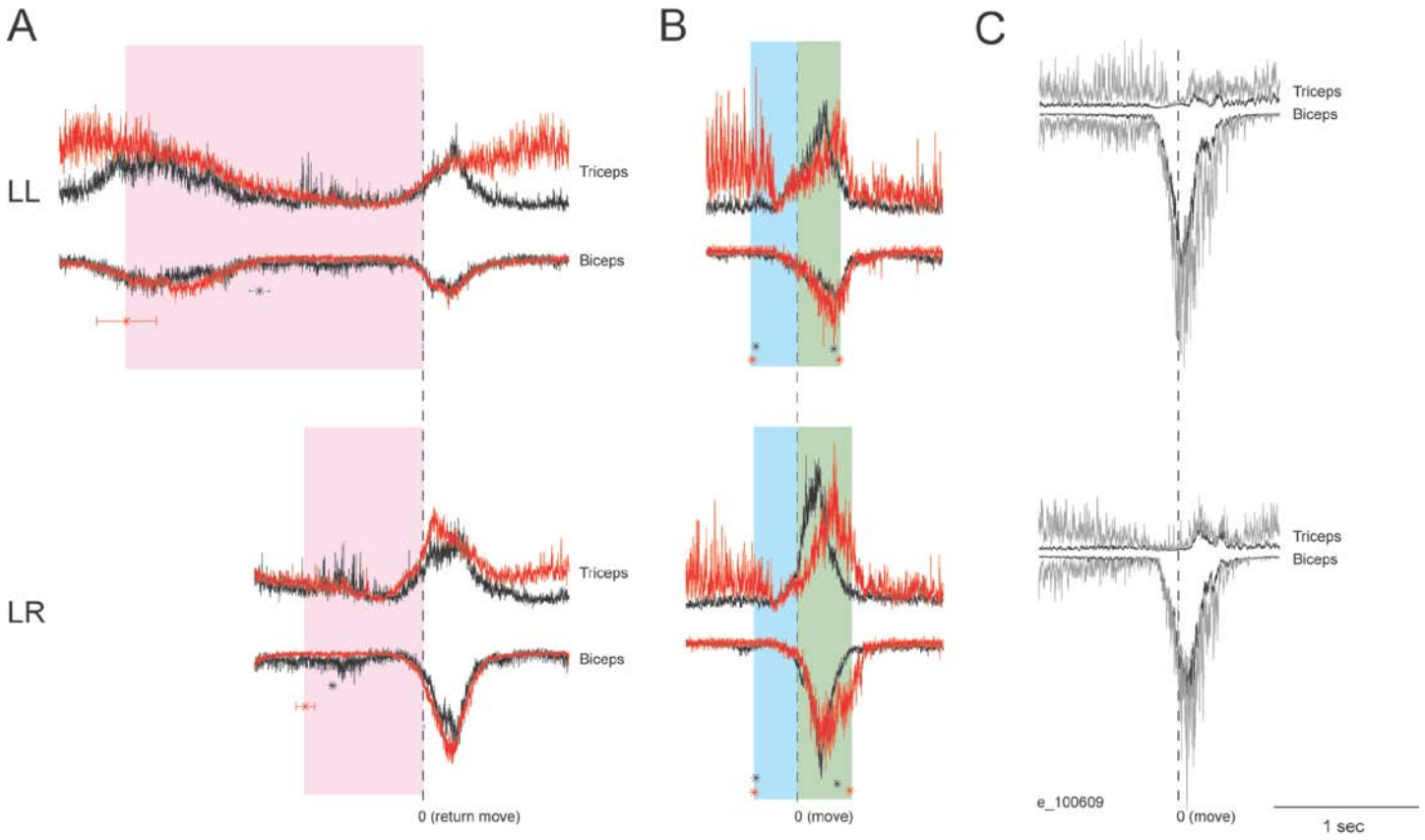

Figure 8: Peri-event EMG activity

This EMG activity was recorded during the same injection as described in Figure 7. The trial type (LL = left hand left target, LR = left hand right target) and muscles recorded are denoted to the right of each trace. A) This figure depicts the average peri-return movement EMG activity pre- (*black*) and post- (*red*) injection. The small error bars indicate the mean (+/- SEM) time at which the reward was given relative to the onset of return movement. The shading represents typical HT (*pink*) periods. Following the injection, there was no change in EMG activity just prior to the return movement. There was, however, a sustained burst of EMG activity following the return movement in both biceps and triceps. This change was more pronounced on LL trials. There was also a burst of EMG activity both pre- and post-injection around the reward time in the LL trials. This likely reflected the latter part of the previous externally-cued movements to the target. Not all of the HTs were very long, so it is likely that the monkey was still moving to the target at this time on many trials. B) This figure depicts the average peri-movement EMG activity before (*black*) and during a 20-trial segment after this injection during which the maximal increase in MTs was observed (*red*). The asterisks to

the left represent the mean (+/-SEM) time of the cue relative to movement onset pre- (*black*) and post- (*red*) injection. The asterisks to the right represent the mean time at which movements were completed pre- (*black*) and during a 20-trial segment post- (*red*) injection. The shading represents typical RT (*blue*) and MT (*green*) periods. Movement-related activity was altered following the injection. There was a slower rate of increase in EMG activity and a smaller and later peak of activity in both the biceps and triceps muscles, more so for left arm to right target trials. There also appeared to be an increase in co-contraction during the movement, as the peaks of muscle activity in biceps and triceps seemed slightly offset in time pre-injection, but became more aligned in time post-injection. C) Peri-movement EMG activity was not similarly affected by saline infusions.

EMG activity during HT periods (i.e., leading up to the initiation of return movements; *pink shading* in Figure 8A) changed very little following this and other cis-flu injections. More specifically, triceps and biceps EMG were not elevated tonically throughout the prolonged HTs. This is contrary to what would be expected if co-contraction was a major contributor to HT prolongations. The initial ramp up of EMG leading to the initiation of return movements was also unchanged following this and other injections. The conspicuous tonic elevation of triceps activity present after completion of return movements (See Figure 8A), and during SPHPs (See Figure 8B), most likely reflected the animal's idiosyncratic tendency to push down on the homekey with more force later in a data collection session. Similar tonic elevations in triceps activity were also observed following saline injections (See Figure 8C).

Injection-related increases in MTs were reflected in peri-movement EMG activity. Figure 8B compares the mean pre-injection peri-movement EMG with the peri-movement EMG averaged across a 20-trial segment of the post-injection period during which the maximal change in MTs occurred. As compared to EMG from the pre-injection period, triceps activity during these 20 post-injection trials was characterized by a slower rate of rise during movement and a

later peak in activity (See Figure 8B, *green shading*). Consistent with the effects of cis-flu on MTs (See Figure 7C), these EMG changes were more prominent for movements to the right (contralateral) target (*LR* in Figure 8B). Finally, there appeared to be increased co-contraction during these externally-cued movements. Specifically, the peak activation of biceps and triceps muscles was slightly offset in time before the injection, but became more aligned in time post-injection.

2.2.3 Frequency and magnitude of behavioral effects

Fifteen of twenty-six cis-flu injections induced some behavioral effect (prolonged HTs, RTs, and/or MTs; according to the χ^2 test; See Figure 9A and Table 1).

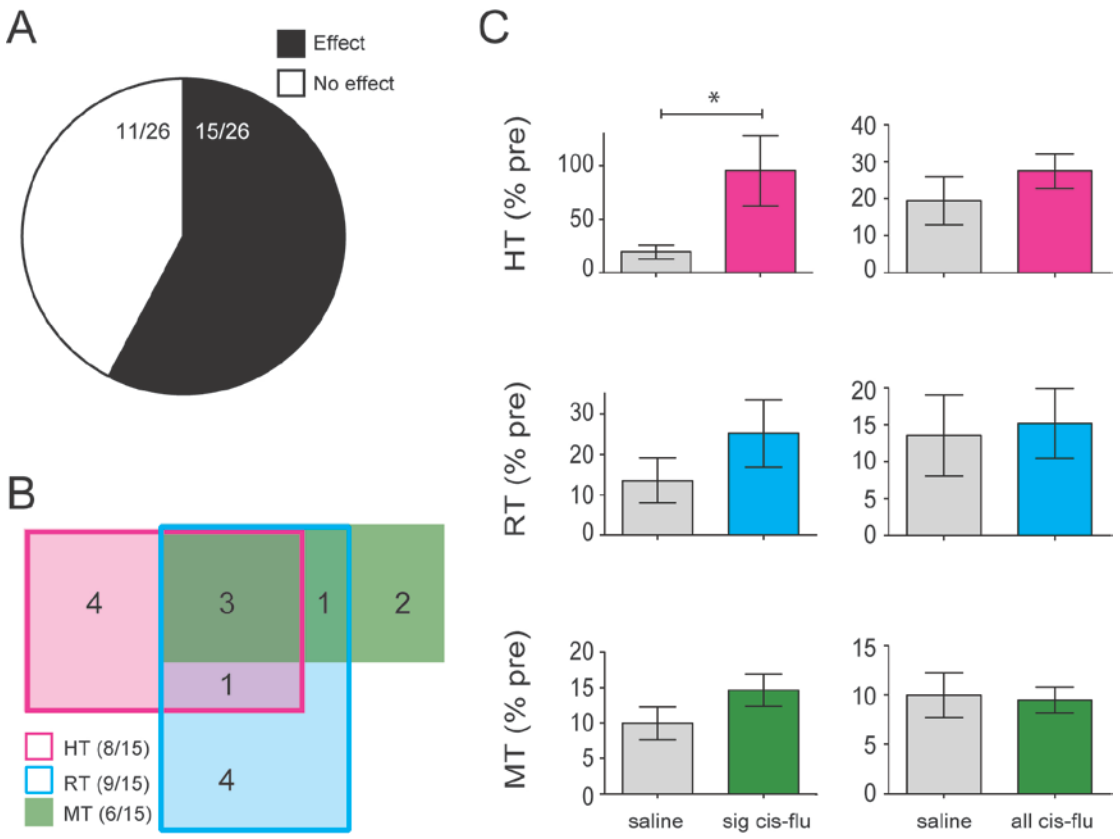

Figure 9: Summary of behavioral effects

This figure represents a summary of the size and magnitude of behavioral effects observed. A) A pie chart represents the fraction of cis-flu injections that elicited an effect. B) A Venn diagram depicts the number of injections that induced one or multiple effects. RT (*blue rectangle*) and HT effects (*pink rectangle*) frequently occurred alone. In comparison, MT effects (*green filled rectangle*) did not frequently occur alone. Sometimes, all 3 effects were elicited, but not very often (*overlap of pink, green and blue*). C) The magnitude of HT effects was greater than the magnitude of saline-induced changes in HTs (*, $p < 0.05$). The magnitude of significant RT and MT effects was not different from saline-induced changes in RTs and MTs, respectively (*first column*). Across all injections, mean injection-related changes in HT, RT, and MT were not different from mean saline-induced changes (*second column*).

Of the 15 injections that induced behavioral effects, 8 injections prolonged HTs, 9 injections prolonged RTs, and 6 prolonged MTs (See Figure 9B). The majority of behavioral

effects we observed (13/15 cases, 87% of effects) consisted of changes in HTs and/or RTs. Only rarely (2/15 cases, 13% of effects) did an injection lengthen MTs alone. Injections of saline never prolonged HTs or RTs, although one saline injection did prolong MTs. Overall, akinetic-like behavioral effects (lengthened HTs and/or RTs) were observed more than twice as frequently as bradykinetic-like effects (long MTs; $p < 0.05$, $\chi^2 = 4.1$).

The magnitude of HT effects following cis-flu infusions (95 +/- 33 % increase; mean +/- SEM) was much greater than the magnitude of change in HTs that occurred following saline injections (19 +/- 6 % increase; $p < 0.05$, Mann Whitney test; See Figure 9C). The magnitude of RT effects (25 +/- 8% increase) was not different from the magnitude of saline-induced changes in RTs (14 +/- 5% increase; $p > 0.05$, See Figure 9C). Similarly, the magnitude of MT effects (15 +/- 2% increase) was not significantly different from the magnitude of saline-induced changes in MTs (10 +/- 2% increase; $p > 0.05$). When averaging across all infusions, HTs were prolonged by 28 +/- 18%, RTs were prolonged by 15 +/- 5%, and MTs were prolonged by 10 +/- 1%. None of these changes were different from saline injections ($p > 0.05$).

In summary, intrastriatal cis-flu injections slowed movement initiation (HTs and RTs) more frequently and to a greater degree than movement execution (MTs). Furthermore, the initiation of non-cued non-rewarded movements (measured as a lengthening of HTs) was affected more severely than the initiation of externally-cued movements (measured as a lengthening of RTs).

2.2.4 Behavioral abnormalities were induced independently

On many occasions, an injection affected one of the three measures of task performance without affecting the other two (See Figure 9B). In other cases, when multiple effects were induced by

the same infusion, they often emerged with different latencies and severity (for an example, compare Figure 7A-C). Furthermore, increases in HT and RT, both ostensible measures of akinesia, were often induced alone. Thus, qualitative observations suggested that cis-flu injections could affect HTs, RTs, and MTs independently.

Consistent with that idea, we found no correlation between the magnitude of change in HTs and RTs or between the magnitude of change in HTs and MTs ($p > 0.05$). Interestingly, however, injection-related changes in RTs and MTs were modestly correlated across injections, solely for the arm contralateral to the site of infusion ($p < 0.05$; Spearman R = 0.56; See Table 2).

Table 2: Relationship between the magnitudes of injection-related changes in behavior

	HT/RT	HT/MT	RT/MT
Contralateral arm	p = 0.3, R = 0.2	p = 0.27, R= 0.22	p = 0.003, R = 0.56 *
Ipsilateral arm	p = 0.9, R = -0.03	p = 0.75, R = 0.07	p = 0.56, R = 0.13

Separate correlations between the magnitudes of injection-related changes in specific behavioral measures are summarized in this table. There was only a significant relationship between the magnitude of injection-related changes in RT and MT in the contralateral arm.

Therefore, while it was possible for cis-flu to affect any one of the three measures of task performance by itself, the magnitudes of change in RTs and MTs were roughly correlated with each other and independent of changes in HTs.

2.2.5 Anatomical distribution of injections with behavioral effects

We found no clear relationship between the location of injections and the patterns of behavioral deficits induced (See Figure 10).

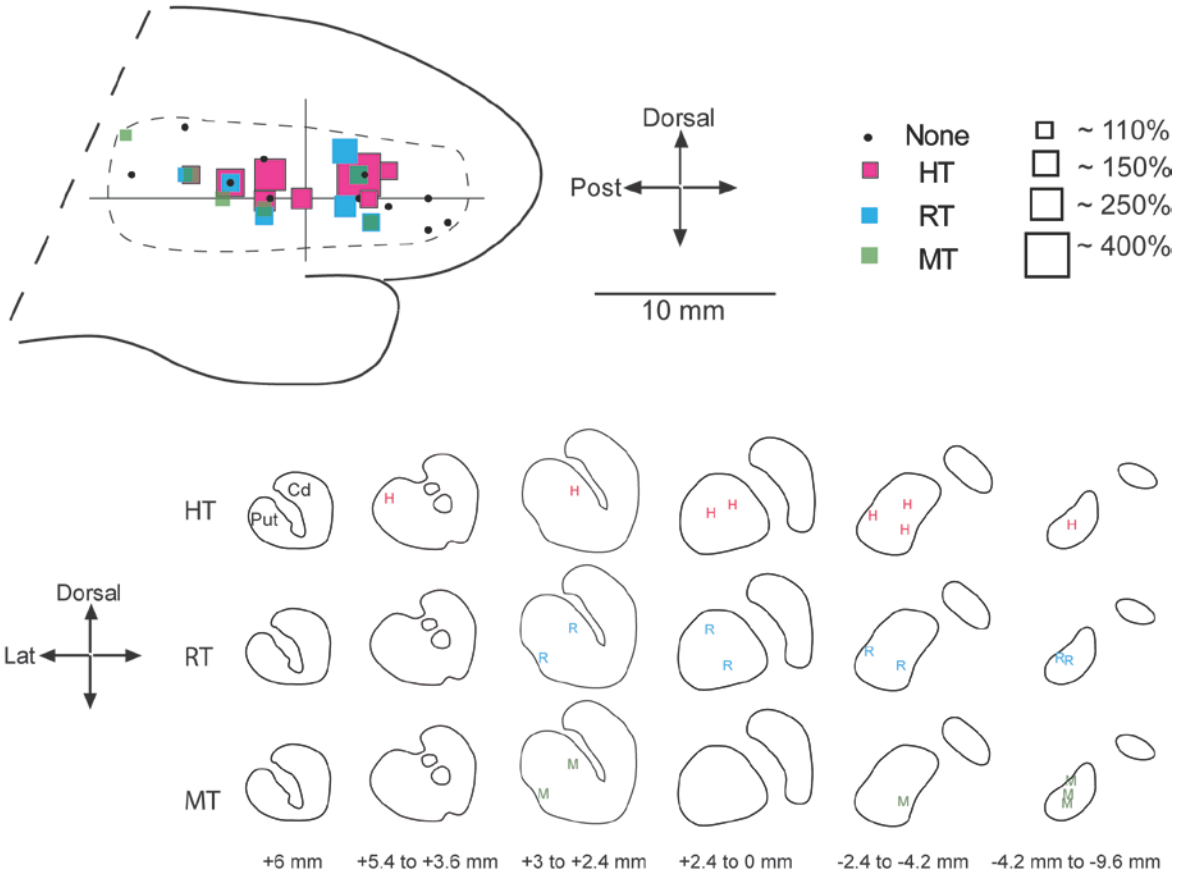

Figure 10: Topography of injection effects

An anatomical reconstruction of injection sites and their effects, collapsed across the sagittal plane. Each injection site is represented by a square that is color-coded based on the type of effect and scaled in size based on the magnitude of the effect (*top*). Significant effects on HT, RT, or MT are depicted on reconstructed coronal planes of the striatum, presented from anterior to posterior (*bottom*). The distance range below each figure represents the AP planes collapsed into that figure (as mm anterior (+) or posterior (-) to the anterior

commissure). Cd = caudate and Put = putamen. There was no obvious topographical organization of effects, although MT effects tended to be more posterior.

2.2.6 No evidence of extinction or general impairment

Previous reports suggest that intra-striatal infusions of cis-flupenthixol selectively impair food-rewarded operant responding (Beninger et al., 1993). We found no evidence that cis-flu injections reduced the rewarding nature of food rewards or reduced an animal's willingness or motivation to perform the operant task. When cis-flu was injected into Monkey D's left putamen, the monkey performed an average of 714 +/- 41 trials (mean +/- SEM) before reaching satiation. When saline was infused into the same hemisphere, the monkey performed only 519 trials. He therefore completed more trials on cis-flu infusion days than on a saline infusion day. The same monkey performed 1093 +/- 92 trials when cis-flu was injected into his right hemisphere, similar to the 1094 trials he performed when saline was infused into the same hemisphere. Similarly, monkey E worked an average of 1227 +/- 29 trials on cis-flu injection days, similar to the 1230 trials she completed when saline was infused into the same hemisphere. In summary, both animals performed comparable numbers of trials on saline and cis-flu infusion days, providing no evidence that intra-putamenal cis-flu injections reduced an animal's general motivation, attentiveness, or willingness to perform an operant task for food reward.

We also found no evidence that cis-flu injections reduced the rewarding nature of food rewards or reduced an animal's willingness or motivation to perform the operant task. Consistent with that interpretation, cis-flu primarily affected performance with the contralateral arm. Both the preponderance of behavioral effects and the strongest behavioral effects appeared for reaches with the arm contralateral to the site of infusion. Specifically, 11/15 injections that

induced significant effects selectively affected the contralateral arm, while 2/15 selectively affected the ipsilateral arm. Finally, 2/15 induced bilateral effects.

2.3 DISCUSSION

For decades, the pathophysiology of PD has largely been attributed to striatal DA loss. It is unclear, however, whether simple under-activation of striatal DA receptors is sufficient to induce parkinsonian signs. Current models of PD do not rule out the importance of chronic striatal DA depletion, extrastriatal DA loss, degeneration of SNpc neurons, and/or changes in other neurotransmitters in the emergence of parkinsonian signs (See *1.0 Introduction*). Furthermore, other studies that have blocked DA receptors in the striatum reported no evidence of parkinsonian signs (Beninger et al., 1993; Nakamura and Hikosaka, 2006). Therefore, the importance of striatal DA loss in PD remains unclear. Given current efforts to develop genetic and transplant therapies with the goal of restoring intra-cerebral DA levels (for review, see Obeso et al., 2010), the specific role of striatal DA loss in PD must be defined.

2.3.1 Intrastriatal DA receptor blockade is sufficient to induce parkinsonian signs

Intrastriatal infusions of DA antagonists provide one approach to determine whether striatal DA loss is sufficient to cause parkinsonian signs. Intrastriatal infusions of DA receptor antagonists are known to elicit catalepsy in rodents (Amalric and Koob, 1989; Ellenbroek et al., 1985; Hauber et al., 2001; Kaur et al., 1997; Salamone et al., 1998; Yoshida et al., 1994). However, the catelptic state is characterized by a nonspecific combination of abnormal posturing and

immobility, making it difficult to determine whether it reflects akinesia or bradykinesia. When Beninger et al. (1993) performed intrastriatal infusions of cis-flu into the rat caudate-putamen, however, they only reported a time-dependent decline in operant responding that resembled extinction, without any evidence of parkinsonian signs. We know of only one previous report on the behavioral effects of DA antagonists infused into the striatum of monkeys (Nakamura and Hikosaka, 2006). In that study, intra-caudate infusions altered the normal variation of oculomotor RTs with the size of a reward, but did not elicit overt signs of parkinsonism. Because these infusions were performed in the oculomotor striatum, however, they may have induced oculomotor signs of parkinsonism that have been reported in PD patients, such as impairments of saccade suppression (Chan et al., 2005) and smooth pursuit (Shibasaki et al., 1979; White et al., 1983), that were not analyzed.

In contrast to those studies, we observed that intra-striatal cis-flu infusions induced specific motor impairments that were reminiscent of parkinsonian signs. We found no evidence that cis-flu injections into the putamen had a global impact on the rewarding nature of food rewards or reduced an animal's general willingness, attentiveness, or motivation to perform the operant task. In support of that view, both animals performed comparable numbers of trials on saline and cis-flu infusion days. Furthermore, cis-flu infusions seldom (2/15 significant effects) affected task performance equally in both arms (i.e., contralateral and ipsilateral to the site of injection) as would be expected if those effects were mediated by a general impairment of motivation, reward processing or attention. Rather, cis-flu injections primarily affected performance of the contralateral arm, consistent with the idea that these were motor impairments.

The disparities between our findings and previous reports could be attributable to differences in injection locations [i.e., putamen here versus caudate and nucleus accumbens in

previous studies (Beninger et al., 1993; Nakamura and Hikosaka, 2006)], species, tasks, and how behavior was measured. More specifically, compared to the intra-caudate infusions performed previously in macaques (Nakamura and Hikosaka, 2006), the infusions here were of larger volume, affected both major classes of DA receptors in the same infusion, and targeted the putamen. Furthermore, the animals here performed a task designed to measure specific motor deficits relevant to parkinsonism, unlike the oculomotor RT task (Nakamura and Hikosaka, 2006) or the task requiring lever pressing for food used in rodents (Beninger et al., 1993).

2.3.2 Types and magnitude of behavioral effects induced by striatal DA receptor blockade

Movement initiation was affected more frequently and severely by intra-striatal DA receptor blockade than movement execution. Interestingly, movement initiation was affected more severely when movements had to be initiated in the absence of direct exteroceptive cues and immediate future rewards. Similarly, PD patients are particularly impaired at initiating and executing internally-generated movements (Cooke et al., 1978; Flowers, 1976; Morris et al., 1996; Oliveira et al., 1997). The contribution of this specific impairment to overall disability in PD is highlighted by the success of assistive technologies for PD patients that provide external sensory cues during activities that would otherwise require self-initiated movements (e.g., Laser Cane and U-step walker from In-Step Mobility Products, Inc.).

The specific mechanisms that mediate this preferential impairment are unknown, but the explanation is likely to fall within one or a combination of two general hypotheses: 1) degradation of a normal function of the BG; or 2) dysfunction of BG-recipient cortical or brainstem regions, potentially caused by aberrant output from the BG. Consistent with the first hypothesis, functional imaging studies have reported enhanced activation in the BG during the

performance of self-initiated movements (Cunnington et al., 2002; Taniwaki et al., 2003) and relative underactivation of the same circuits in PD patients (Jahanshahi et al., 1995). Single unit recording in neurologically normal animals has shown that self-initiated movements are preceded by slow ramping activity in the striatum (Schultz and Romo, 1992). Moreover, Lee and Assad (2003) reported that higher levels of striatal preparatory activity were correlated with faster response times, leading them to propose that loss of striatal DA in PD may slow the initiation of self-generated movements by reducing the rate of preparatory ramping of activity. One difficulty with this hypothesis, however, is the consistent observation that self-initiated movement is not impaired following temporary or permanent inactivation of the GPi (Fine et al., 2000; Mink and Thach, 1991b; Obeso et al., 2009; Vitek et al., 2003).

An alternative view is that abnormal inhibitory output from the parkinsonian BG disrupts the function of cortical areas known to be important for self-initiated movement (Jahanshahi et al., 1995; Playford et al., 1992). Mesial premotor cortical areas, including the supplementary motor area (SMA), are strongly innervated by BG outflow (Akkal et al., 2007; Schell and Strick, 1984) and become markedly under-activated in PD patients (Jahanshahi et al., 1995; Playford et al., 1992; Wu et al., 2010). The activity of these regions is normalized in response to anti-parkinsonian therapies (Grafton et al., 2006; Grafton et al., 1995; Jenkins et al., 1992). It is interesting to note, therefore, that the mesial premotor areas are thought to be particularly important for the initiation of movement in the absence of external sensory cues (Tanji and Hoshi, 2008). Thus, underactivation of these areas following striatal DA loss could impair the initiation of self-generated movements.

2.3.3 EMG

In the example shown here, there was no obvious physiologic correlate of prolonged HTs in the biceps or triceps muscles. Specifically, there was no evidence of co-contraction or increased tonic muscle activity during HTs following this cis-flu injection. We did observe, however, a disorganized and sustained increase in EMG activity after the initiation of return movements. Additionally, return movements of the left arm from the right target appeared to be associated with a slightly greater peak EMG activity in the triceps that occurred earlier relative to movement onset. The indication of these two findings is unclear, especially since we could not analyze return movements due to excessive variability (see *Methods*).

We also found no evidence that prolonged RTs could be attributable to increased co-contraction of biceps and triceps. Instead, increased co-contraction of antagonistic muscle pairs seemed to be associated with prolonged MTs. In both muscles, longer MTs after cis-flu injections were also associated with a reduced rate of increase in EMG activity, and a smaller peak of EMG activity that occurred later after movement onset. This is consistent with studies in PD patients suggesting that bradykinesia is related to a reduced rate of increase in peak EMG activity (Godaux et al., 1992) and a reduction in the first agonist burst (Berardelli et al., 1996a; Doudet et al., 1990). In contrast to other studies (Hallett and Khoshbin, 1980), however, we did not observe multiple cycles of alternating biceps and triceps activity during movement execution.

2.3.4 Specific parkinsonian signs may have distinct pathophysiologies

We were able to elicit akinetic and bradykinetic signs independently. Many injections selectively prolonged RTs, HTs, or MTs. Even when more than one sign was elicited after an

injection, the timing and severity of each effect was often quite different. The degree to which RTs and MTs increased after each injection was closely related, but was independent of the degree to which HTs increased. This relationship between RTs and MTs was only observed for movements of the arm contralateral to the infusion site, suggesting that both effects arose from a common pathophysiologic mechanism and that that mechanism was lateralized.

Given this relationship between changes in the magnitude of RTs and MTs, it seems paradoxical that RT and MT effects could be observed alone after many cis-flu injections. This apparent discrepancy is resolved when considering the different methods by which we defined the significance and magnitude of effects. Specifically, some injections induced RT effects without MT effects (and vice versa), demonstrating that some injections preferentially increased the frequency of trials with prolonged RTs or MTs. However, the overall magnitude of change in RTs and MTs across all injections still remained closely related. In other words, the frequency of trials with prolonged RTs or MTs could be selectively increased in a given injection, while the overall magnitude of injection-related changes in RTs and MTs across injections remained closely related.

Our findings are consistent with other studies suggesting that specific parkinsonian signs may be induced independently. Specifically, lesions of the macaque putamen impair the initiation of movements, without affecting movement execution (Nixon and Passingham, 1998). In PD patients, the magnitude of levodopa-induced improvements in gait freezing is unrelated to the magnitude of improvement in bradykinesia (Bartels et al., 2003). Furthermore, specific parkinsonian signs tend to cluster together in PD patients, such that PD can be divided into subtypes such as a postural instability/gait difficulty or tremor dominant subtype (Doder et al., 2003; Zetusky and Jankovic, 1985). The time-course and severity of sub-groups of parkinsonian

signs also vary independently between patients (Evarts et al., 1981; Jordan et al., 1992; Meyer, 1982; Nieuwboer et al., 1998; Selikhova et al., 2009; Temperli et al., 2003; Zetusky and Jankovic, 1985), lending further support to the idea that different signs have separate pathophysiologic substrates. Finally, the fact that PD patients exhibit greater difficulty initiating self-generated movements than externally-cued movements and even use external cues to assist in initiating self-generated movements suggest that impairments in the initiation of self-generated and externally-cued movements are generated by independent mechanisms.

Our findings could suggest that striatal DA may be preferentially involved in the initiation of self-generated than externally-cued movements. These findings are also consistent with the hypothesis that self-initiated movements were slowed due to disruptions in cortical activity secondary to striatal DA loss. It is possible, however, that externally-cued movements were less affected due to the emergence of task-specific compensatory mechanisms. In sum, our data support the idea that the initiation and execution of self-initiated and externally-cued movements are independently affected in PD, and impairments in each may be attributable to unique pathophysiologic substrates.

2.3.5 Other Considerations

These results do not rule out the possibility that DA loss from extra-striatal structures may also contribute to the genesis of parkinsonian signs. Here, we observed akinetic signs (long RTs and/or HTs) with greater frequency and severity than bradykinetic signs (long MTs). This suggests that extrastriatal DA loss, chronic striatal DA loss, more widespread DA loss, chronic DA loss, non-dopaminergic pathologic changes or other mechanisms might play a more important role in the genesis of bradykinesia, while acute striatal DA loss appears to be an

adequate trigger for the pathophysiologic cascade that leads to akinetic parkinsonian signs. Regardless of the specific mechanism, this disparity suggests separate pathophysiologic mechanisms for akinesia and bradykinesia. Possibly, DA loss in other brain regions (e.g., cortex) may also contribute to bradykinesia (Gaspar et al., 1991; Scatton et al., 1983; Scatton et al., 1982). Injections of DA antagonists into the dorsal premotor cortex have been shown to impair RTs and MTs in macaques (Sawaguchi, 1997). Degeneration of non-dopaminergic neurons may also play a role in the development of bradykinesia. For example, pyramidal tract neurons in the pre-supplementary motor area are known to degenerate in PD patients (MacDonald and Halliday, 2002).

2.3.6 Lack of Topographic organization of effects

The striatum is divided into anatomical subdivisions based on projections from distinct cortical areas (Nambu et al., 2002a; Parent and Hazrati, 1995; Takada et al., 1998). Intrastriatal injections of bicuculline have also revealed that the striatum is organized topographically into separate functional regions (Worbe et al., 2009). We therefore expected that the effects of intrastriatal DA receptor blockade would be topographically organized.

Instead, injection sites that induced specific types of effects exhibited no obvious topographical organization. This could be attributable to a variety of factors. Based on our use of convection-enhanced delivery methods and the volume of our infusions, the drug likely diffused into a roughly spherical territory around the injection site ~ 4.6 mm in diameter (Vogelbaum, 2005), which amounts to ~11% of the total volume of the macaque putamen (Harman and Carpenter, 1950). It was therefore likely that we affected many striatal territories with some injections, especially if they were performed near functional boundaries [See Figure 6

in (Worbe et al., 2009) for a depiction of how injections in specific locations could impact either one or multiple functional territories]. Notably, DA receptor blockade in more posterior regions of the striatum often prolonged MTs (See Figure 10), as would be expected based on the anatomical subdivisions of the striatum (Nambu et al., 2002a; Parent and Hazrati, 1995; Takada et al., 1998). It was surprising, however, that infusions into the microexciteable arm area of the putamen (the definitive motor arm area) did not cause slowing of movement. While we did observe some akinetic effects after infusions into the microexciteable arm area of the putamen, those effects were not among the most severe.

These observations bring into question current hypotheses suggesting that DA loss in the "motor" putamen is the primary cause of parkinsonian signs (Kish et al., 1988), especially of bradykinesia. It is also noteworthy that the most severe akinetic effects were elicited by infusions into anterior/medial regions of the putamen, regions considered part of the associative functional circuit (Alexander et al., 1990; Worbe et al., 2009). Our results indicate that components of the parkinsonian state can be elicited by transient interruption of DA signaling at a variety of putamenal locations, including sites in motor and associative regions. The absence of a clear topography suggests that the relationship between BG functional circuits, sites of DA loss, and resulting parkinsonian signs is more complicated than is often hypothesized (Alexander et al., 1990).

2.3.7 Limitations and Potential Confounds

Many of the infusions (42%) elicited no apparent behavioral effect. Other studies have also reported that a large fraction of intrastriatal pharmacologic manipulations in macaques failed to produce detectable behavioral effects (McCairn et al., 2009; Worbe et al., 2009). It is possible

that some infusions altered behaviors that were not measurable by the arm movement task (e.g., changes in facial or leg motor control or in higher cognitive functions (Cooper et al., 1991; Dubois and Pillon, 1997; Lees and Smith, 1983; Owen et al., 1992; Pessiglione et al., 2003). Two of the 26 injections performed here were repeat injections, performed in the same location as prior injections. Only one of these two injections elicited the same response as the earlier injection in the same location, and the effect was much weaker. This variability could be attributable to a variety of causes.

Infusing DA antagonists into the striatum may have induced compensatory responses that attenuated the behavioral response to future infusions within the same or nearby regions. Compensatory responses might include glial proliferation, increases in DA synthesis and release, and/or an up-regulation of DA receptors on MSNs, or interneurons. Behaviorally, these changes would likely have manifest as tardive dystonia-like signs (Stahl, 2002). Another potential source of variability was the possibility that tissue damage from an earlier cannula track may have provided a low resistance path for the solution, allowing for reflux or diffusion of drug away from the injection site (Alexander et al., 2010).

One additional point that may be seen as a limitation is that we were unable to elicit tremor. This is not surprising, however, because resting tremor is rarely observed in macaque models of parkinsonism, even with near total loss of putamenal DA (Bergman et al., 1994; Eberling et al., 2000). Importantly, we were able to elicit other cardinal signs of parkinsonism independently and with differing degrees of severity.

2.3.8 Conclusions

Our results suggest that acute, focal striatal DA receptor blockade is sufficient to induce selective parkinsonian motor deficits. Specifically, the intra-striatal infusion of a D1- and D2-receptor antagonist in awake, behaving macaques was found to impair the initiation of movements more than the execution of movements. Paralleling observations in PD patients, the initiation of self-generated movements was impaired more severely than the initiation of externally-cued movements. Additionally, specific parkinsonian signs were often induced independently, thereby suggesting the existence of distinct pathophysiologic substrates. Further research on this topic may lead to the development of therapies that target specific PD signs. Finally, the infrequent and weak effects of striatal cis-flu on movement speed (reflecting bradykinesia) accentuate the potential importance of extra-striatal DA loss in the genesis of that specific sign.

3.0 NEUROPHYSIOLOGIC EFFECTS OF INTRASTRIATAL DA RECEPTOR BLOCKADE

3.1 INTRODUCTION

According to the classic rate model, DA depletion in the striatum decreases GPe activity and increases GPi activity, which inhibits M1 activity, thereby impairing movement (Albin et al., 1989; DeLong, 1990; Miller and DeLong, 1987; Wichmann and DeLong, 1996). Consistent with this model, there is evidence that GPi firing rates are elevated in both MPTP-treated parkinsonian macaques (Filion and Tremblay, 1991; Miller and DeLong, 1987) and in PD patients (Hutchison et al., 1994; Sterio et al., 1994). Others, however, report no change in BG firing rates (Raz et al., 2000) and suggest that abnormal activity patterns in the BG may play a more important role in the pathophysiology of parkinsonian signs. Increases in bursting activity (Bergman et al., 1994; Boraud et al., 1996; Boraud et al., 1998; Boraud et al., 2000; Filion and Tremblay, 1991; Hutchison et al., 1997), oscillations (Bergman et al., 1994; Filion and Tremblay, 1991; Hutchison et al., 1997; Levy et al., 2002b; Miller and DeLong, 1988; Nini et al., 1995), synchrony (Goldberg et al., 2002; Hurtado et al., 1999; Levy et al., 2000; Nini et al., 1995; Pessiglione et al., 2005; Raz et al., 2000), and reduced selectivity of neuronal activity (Bergman et al., 1998; Filion et al., 1994; Nini et al., 1995) in the BG are all associated with the parkinsonian state. The cause and pathophysiologic relevance of these changes remains unclear.

Like the activity in the parkinsonian BG, the activity in M1 becomes more synchronous and bursty in the parkinsonian state. M1 activity also becomes less specific to passive limb movements (Goldberg et al., 2002) and is characterized by reduced directional tuning and peri-movement discharge in the parkinsonian state (Desmurget and Turner, 2008). These changes may be more important in the development of parkinsonian signs than alterations in firing rate, as reduced M1 firing rates in have only been reported in some studies of parkinsonian animals (Parr-Brownlie and Hyland, 2005; Pasquereau and Turner, 2010), but not others (Doudet et al., 1990; Goldberg et al., 2002).

It is difficult to demarcate which changes in neuronal activity might contribute to the development of parkinsonian signs and which might reflect pathologic or compensatory responses to striatal DA loss without causing parkinsonian signs. Thus, the pathophysiologic mechanism whereby striatal DA loss might cause parkinsonian signs remains unclear. Striatal DA loss may induce parkinsonian signs by degrading the normal functioning of the BG; or by causing dysfunction of BG-recipient cortical or brainstem regions. Presently, the effects of striatal DA loss alone on cortical and BG activity remain unknown. Here, we test the fundamental hypothesis that acute striatal DA loss is sufficient to cause abnormal changes in cortical and BG activity that are associated with the parkinsonian state.

3.2 METHODS

3.2.1 Animals and Task

Two monkeys (*Macaca mulatta*; D, male ~7.5 kg; E, female ~6 kg) participated in the study. All aspects of animal care were in accord with the ‘‘Guide for the Care and Use of Laboratory Animals’’ (National Academy Press, 1996), and all procedures were approved by the institutional animal care and use committee. The animals used in this study were the same animals used in the previous study (See *Section 2.0*) and performed the same simple reaching task described previously in detail.

3.2.2 Surgical Procedures

Many of these methods have been described previously (see Section 2.0 and Desmurget and Turner, 2008). Animals were prepared for microinjections using aseptic technique under isoflurane anesthesia. Two cylindrical titanium recording chambers (18 mm ID) were affixed to the skull over a craniotomy in stereotaxic coordinates to allow transdural access to the right and left putamen and globus pallidus externa and interna from a coronal approach (for detailed description of surgical techniques, see Turner and Anderson, 1997). The chamber was fixed to the skull with bone screws and dental acrylic. Bolts embedded in the acrylic allowed fixation of the head.

After implantation of a recording chamber, the cortex, GPe, and GPi were delineated by acquiring neurophysiologic information about anatomical boundaries and neuronal responses to movement of contralateral limbs. The motor putamen was defined by areas that evoked reliable

muscle contractions in the leg, arm, or face in response to microstimulation (300-400 Hz, 40-60 µA, 10 pulses, 200-300 µsec pulse duration). This information was merged onto each monkey's MRI using Cicerone (Miocinovic et al., 2007). Following surgery, animals were given prophylactic antibiotics and analgesic medication. After recovery from surgery, animals resumed behavioral testing.

3.2.3 Micro-injections

These methods are described in detail in *Section 2.1.4. Microinjections*. Briefly, cis-flupenthixol (10 µl) was infused at sites throughout the anterior-posterior extent of the putamen using convection-enhanced delivery, or bulk flow. Injection sites were pre-determined by overlaying chamber positions and electrophysiologically-derived maps onto each monkey's MRI using Cicerone, a customized software program (Miocinovic et al., 2007).

Once a week, infusions of either cis-flupenthixol (cis-flu; D1 and D2 antagonist, Sigma-Aldrich) ^{or} 0.9% sterile saline were performed. Before behavioral recording began, the tip of the injection cannula was first lowered to one pre-determined injection site through a dura-piercing guide tube placed inside a grid. During seventeen cis-flu and two saline injections, three to four electrodes (~0.5 mm apart) were then lowered into the cortex, GPe, and GPi through one large guide tube fixed in a separate hole in the same grid. This permitted the recording of simultaneous neuronal activity from up to three structures throughout injections (Figure 11). During one injection, electrodes were lowered through a separate chamber via a sagittal approach rather than through the same grid.

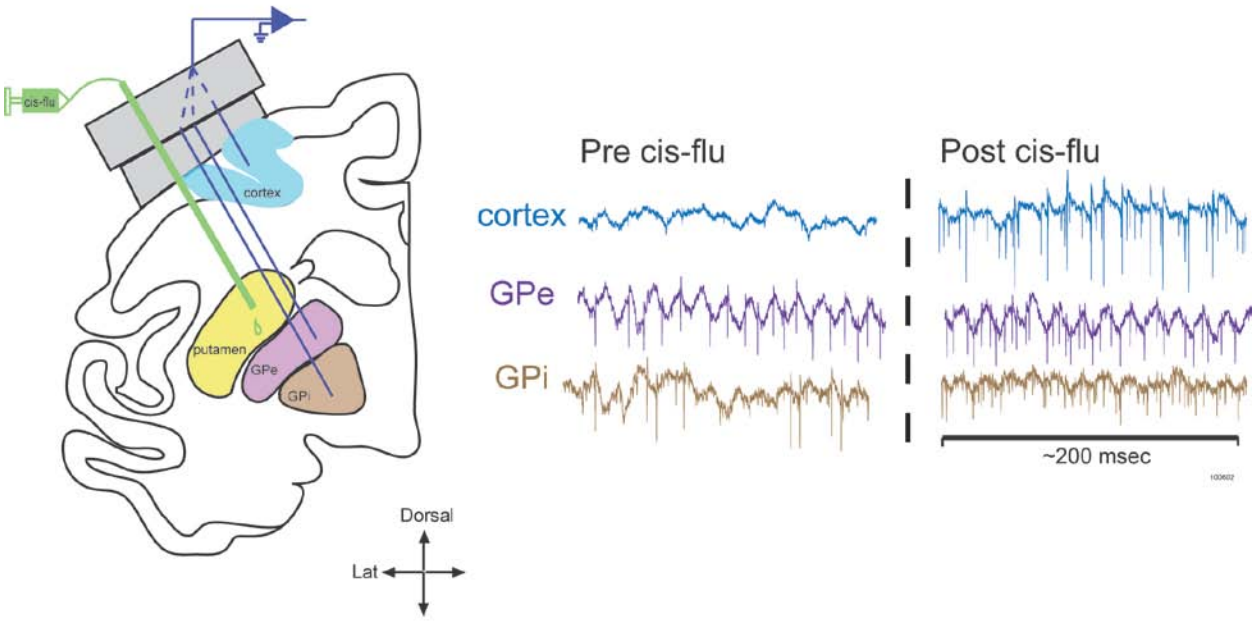

Figure 11: Recording setup

The diagram on the left illustrates a coronal view of the technical approach used to perform simultaneous recording and injection experiments. A micro-injection cannula was lowered into the putamen while 3-4 electrodes were lowered into the cortex, GPe, and/or GPi through the same chamber. An example of raw, continuous data collected simultaneously from three separate electrodes in three separate structures before and after an injection is displayed on the right.

Behavioral data from the task was collected before (15-20 min), during (10.5 min), and after each injection (total ~1 hr 30 min), until the monkey was satiated. The cannula and electrodes remained in the brain throughout all recordings. On saline injection days, 10.5 µl of sterile saline was infused into the putamen using the same apparatus. Based on the large volume of solution infused, we predicted the solution would diffuse ~2.3 mm away from the injection site, creating a sphere centered at the injection site ~ 4.6 mm in diameter (Vogelbaum, 2005).

3.2.4 Data Acquisition

Transdural extracellular recording was performed simultaneously from one to four glass-coated platinum/iridium microelectrodes (0.5-1 megohm impedance) mounted into a motorized microdrive (4-channel MT, AlphaOmega). The electrodes were advanced through separate guide tubes nested together inside one larger guide tube that rested on the surface of the dura. The distance between electrodes was approximately 0.5mm. During each injection, at least one electrode was targeted for each of the following structures: cortex, GPe, and GPi. Due to the tight spacing between electrodes, it was rarely possible to record simultaneously from the motor territories of all three structures. A particular location was chosen as a recording site during an injection if 1) cortical neurons in that region had previously responded to active and/or passive movement of the arm and 2) the location appeared to be in premotor or motor cortex when reconstructed onto the monkey's MRI using Cicerone.

Electrodes were advanced into the brain using customized computer software (AlphaOmega) that allowed independent control of each channel. Continuous neuronal data was sampled from each of four channels at ~24 kHz. All of the data were collected using a real-time processor (RZ2, Tucker-Davis Technologies). All single neuron data were sorted offline using cluster detection in principle components space (OffLine Sorter, Plexon Inc.) Spike times were used to evaluate firing rates and patterns. A separate video capture setup (Nuvico EV-4000) recorded the animal’s general behavior and movement while performing the task. Behavioral events were stored with 40 μsec accuracy.

3.2.5 Behavioral Analyses

The behavioral data and the corresponding analyses are identical to those described previously (See *2.1.5. Behavioral Analyses*). Briefly, significant cis-flu related effects on hold times (HT), reaction times (RT), and movement times (MT) were determined using chi-squared analyses. To determine the magnitude of change in each behavioral measure, a sliding window of 20 consecutive trials of the same trial type was stepped trial-by-trial through the post-injection data. The within-window mean of a given behavioral measure was normalized by dividing by that measure's pre-injection mean (i.e., 0% indicated no change from pre-injection). The maximum percent change across all positions of the sliding window was taken to represent the magnitude of an injection-related change in behavior.

3.2.6 Neuronal Analyses

Digitized spike trains were imported into off-line spike sorting software (Plexon Inc., Dallas TX) for discrimination of single-unit action potentials by cluster-cutting in principal components space. This software generated a record of the time of occurrence (reduced to millisecond accuracy) for each action potential waveform detected. The spike times were used to calculate discharge rate and bursting. Analyses were performed in the Matlab computing environment (The Mathworks, Natick, Massachusetts). Average firing rates and burstiness were extracted during the resting hold period of the task, during which the animals' hands were resting on the two homekeys while the animal awaited an instruction. This was thought to reflect neuronal activity during a resting, but alert behavioral state. The first 0.5 seconds of each hold period was removed due to greater variability of the animals' state during this period of time.

Bursts were detected using the Poisson Surprise Method (Legendy and Salcman, 1985; Wichmann et al., 1999). Each period of increased neuronal discharge was assigned a surprise value (*S*) that quantified the likelihood that this period of activity was a burst (i.e., a discrete period of elevated firing rate), rather than part of the neuron's ongoing stochastic firing pattern. The surprise value (*S*) can be represented as:

$$S = -log(P)$$

where *P* is the probability that a Poisson spike train with the same mean firing rate would generate more spikes than emitted by the burst in the same time period. Bursts were identified as a sequence of at least three inter-spike intervals (ISIs) with a Poisson surprise value > 5 ($P < 0.00001$). For each such burst, immediately-adjacent preceding and trailing spikes were added incrementally to maximize the Poisson surprise value. The onset and offset of a burst were set as the times of the first and last spikes of the burst. The overall “burstiness” of a cell was quantified as the fraction of spikes that occurred during bursts relative to the total number of spikes in the cell's recorded spike train.

3.2.7 Average Firing Rates and Burstiness

Average resting firing rates and burstiness of cells were compared before and after cis-flu injections. Due to the long period of time required to inject the total volume of solution (10.5 min), the first 315 seconds of the injection period was considered part of the "pre-injection” period. The beginning of the post-injection period included the last 315 seconds of the injection period. We removed cells that were recorded both pre- and post- injection in this analysis because it is statistically invalid to include paired and unpaired values in the same t-test. Only cells recorded pre- injection or post- injection were chosen to represent the population of cells in

these analyses to maximize the number of cells that could be analyzed, thereby minimizing the likelihood of sampling bias.

As predicted by the rate model, we hypothesized that firing rates in cortex and GPe would decrease. Burstiness was expected to increase in both structures (see *Introduction*). Due to the temporary nature of some drug-related changes in firing rate or burstiness, we used a sliding window of 80 consecutive trials to search for the maximum and minimum burstiness and firing rate, respectively, of each pre- and post-injection period. We chose to look for the maximum burstiness and minimum firing rates due to our initial hypotheses that burstiness would increase and firing rates would decrease. The maximum burstiness and minimum firing rate of each pre- and post- injection period was then averaged across all pre- and post-injection periods for the cortex and GPe separately. It could be argued that searching for the maximal burstiness or minimal firing rate increased the likelihood of finding a significant change in burstiness or firing rate. Importantly, however, the same sliding window approach was used to define burstiness and firing rate during pre-injection periods. One-tailed Mann-Whitney tests were used to compare maximal burstiness and minimal firing rates pre- vs. post- injection.

To ensure that changes in firing rate and burstiness were not simply due to a mechanical effect of the injection, the same analyses were done on data collected during saline injections. Each cell's injection-induced change (%) in burstiness and firing rate was then quantified by dividing the firing rate or burstiness of each cell recorded post-injection by the overall mean firing rate or burstiness of all cells recorded pre-injection. This was done separately for cells recorded during cis-flu injections and cells recorded during saline injections. One-tailed Mann-Whitney tests were then employed to compare saline-induced changes in burstiness and firing rate with cis-flu-induced changes in burstiness and firing rate, respectively. However, there were

not enough GPe cells recorded during saline injections to compare saline-related and cis-flu-related changes in GPe burstiness or firing rate.

3.2.8 Relation of firing rates and burstiness to behavior

To relate changes in resting firing rates and burstiness with changes in behavior, the resting firing rate and burstiness for each trial was correlated with behavioral measures (RT, HT, and MT) from the same trial. Separate Spearman correlations were performed pre- and post-injection for each cell. These correlations yielded a rho value for each combination of neuronal activity measure (firing rate, burstiness) and behavioral measure (RT, MT, HT for each arm) for each cell, pre- or post-injection. Mann-Whitney tests were used to compare Spearman rho values for cells only recorded pre- injection with those only recorded post-injection in each structure.

3.2.9 Peri-event activity

The average peri-movement activity of each cell was determined for outward and return movements. First, we created a peri-movement spike density function for each neuron and arm (i.e., pre-injection left arm, pre-injection right arm, etc). Peri-movement activity was then quantified for each neuron/condition pre- and post- injection separately. Baseline resting activity (acquired during a 200-300 msec window prior to movement) was then subtracted out of each cell's peri-movement activity. Finally, we compared peri-movement activity pre- vs. post-injection across the population of cells using Wilcoxon rank sum tests. Separate comparisons were performed on cortical and GPe cells.

In contrast to the firing rate and burstiness analyses, only cells recorded both pre- and post- injection were included in these peri-movement analyses. Cells that were not recorded throughout injections (both pre- and post-injection) were excluded to eliminate the confound introduced by the fact that different proportions of cells with movement-related activity were likely recorded pre vs. post- injection. While cells recorded pre- or post- injection were not included in this analysis, Mann-Whitney t-tests were used to compare the average peri-movement activity of these cell populations pre- vs. post-injection in separate analyses.

3.2.10 Oscillatory activity

LFP signals from each electrode were examined for changes in α- (8-13 Hz), β-(13-30 Hz), and low gamma (30-100 Hz) oscillatory activity before and after injections. After removing linear trends in the LFP signal, the data was high-pass (5 Hz) and stop-pass (295-305 Hz and 55-65 Hz) filtered using customized Butterworth filters (Matlab). The power spectral density was then computed by applying a Fast Fourier Transform and one-sided Hanning window to the signal. The total power in the α-, β-, and low gamma bands was divided by the total power in the high gamma band (300-500 Hz) to yield the relative power of each frequency band during each resting hold period.

Next, we sought to quantify the injection-related change in the relative power of each frequency band for each electrode and each injection. This was done by dividing the mean relative power of each frequency band across all post-injection resting hold periods in one injection by the mean relative power of each respective frequency band across all pre-injection resting hold periods in the same injection. The drug-induced changes in the relative power of each frequency band were then averaged across each structure (cortex, GPe, or GPi). A structure

was defined as having a significant injection-induced change in a specific frequency band if the standard error of the mean change in a specific frequency band excluded zero.

3.2.11 Spike-triggered LFP activity

LFP is believed to reflect the synaptic activity within a few millimeters of the electrode. It is thought that spiking activity influences LFP activity, but the relationship between LFP activity and spiking activity presently remains unclear. Here, we used spike-triggered averaging of LFP activity to further explore the relationships between spiking activity and LFPs in the cortex and BG and to determine how these relationships might be affected by striatal DA receptor blockade.

First, we correlated spiking activity with fluctuations in the LFP recorded on the same electrode to determine if they were related in time. This provided one measure of synchrony. Specifically, we averaged the LFP activity across a time window from 50 msec before to 50 msec after each spike. One average peri-spike LFP was computed for each cell across the entire pre- or post- injection. If a cell was only recorded pre- or post-injection, only one peri-spike LFP was generated for that cell.

Significant spike-triggered LFP effects were identified by calculating the standard deviation of each average spike-triggered LFP after reversing the LFP activity in time. The standard deviation of this time-reversed average spike-triggered LFP was then multiplied by 3.1 and -3.1 to yield positive and negative significance thresholds for each cell, respectively. A cell was said to have a significant correlation between spiking and LFP activity if the average spike-triggered LFP crossed either significance threshold any time 50 msec before and 50 msec after spike onset. The proportion of cells with significant spike-triggered LFP activity was quantified pre- and post- injection and compared using a chi-squared test. In this way, we could test our

hypothesis that more cells would become synchronized, or time-locked, with LFP activity after intrastriatal DA receptor blockade.

To determine whether spikes became more synchronized with LFP activity at a specific frequency after intrastriatal DA receptor blockade, we computed a power spectrum of each spike-triggered LFP. The total power of spike-triggered LFP activity in the α-, β-, and low gamma frequency bands was divided by the total power in the high gamma frequency band to yield the relative power of each frequency band for each spike-triggered LFP. Mann-Whitney tests were used to determine whether there were any drug-related changes in any of the frequency bands for each structure. This analysis only included cells that were recorded pre- or post-injection, but not both.

3.3 RESULTS

A total of seventeen intrastriatal cis-flu injections were performed across three hemispheres in two monkeys (nine injections across two hemispheres in monkey D, eight injections in one hemisphere in monkey E). In the cortex, eighteen neurons were recorded during a pre-injection period and thirty-two neurons were recorded during a post-injection period. Of these cells, eleven were recorded both pre- and post-injection. The remainder of these cells were recorded either pre- or post-injection, but not both. In the GPe, twenty-one and forty-two neurons were recorded during pre- and post-injection periods, respectively. Fourteen of these neurons were recorded both pre- and post-injection. Finally, four GPi neurons were recorded pre-injection and four GPi neurons were recorded post-injection. Two of these neurons were recorded both pre-

and post-injection. Unfortunately, there were too few GPi cells to perform any statistical analyses on these cells.

Three intrastriatal saline injections were also performed, one in each hemisphere. Neuronal data was recorded from the cortex, GPe, and GPi during two of these saline infusions. In the cortex, two cells were recorded during a pre- saline injection period and six neurons were recorded during a post- saline injection period. Only one of these cells was recorded during a pre- and post-injection period. Two GPe neurons were recorded both pre- and post- saline injection. In the GPi, only one cell was recorded both pre- and post- saline injection. Saline injections were primarily used to ascertain that behavioral effects were specific to cis-flu. While few in number, neuronal recordings during saline injections were also used to determine whether similar changes in neuronal activity were found after cis-flu and saline injections.

3.3.1 Resting firing rates and burstiness

Intrastriatal DA receptor blockade suppressed cortical and GPe firing rates. Specifically, resting firing rates in the cortex decreased from 25 +/- 12 to 5 +/- 1 spk/sec (mean +/- SEM; $p < 0.05$ Mann-Whitney, one-tailed test; See Figure 12).

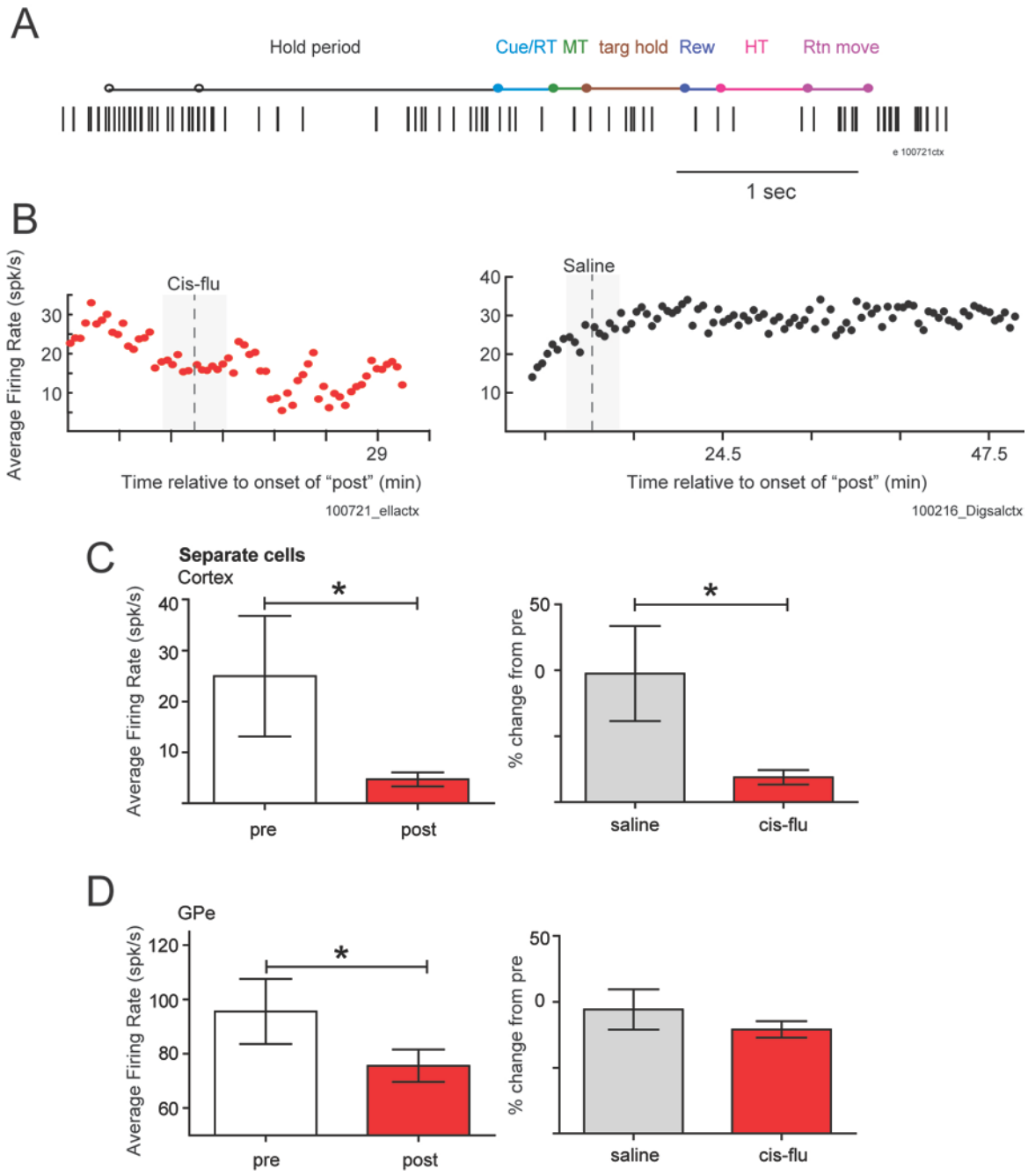

Figure 12: Firing rate changes following injections

A) A rasterplot of a single neuron, aligned in time with separate epochs of the task. For most analyses, only the activity during the resting hold period (*black line*) was examined. The first 0.5 seconds of each resting hold period (*the period between the first two black circles*) was excluded due to behavioral variability during

that time period. B) The mean resting firing rates of one cortical cell (in 20-trial increments) throughout one cis-flu injection (*red*) is depicted on the left. The mean resting firing rates of another cortical cell (in 20-trial increments) throughout a saline injection (*black*) is depicted on the right. C) Mean firing rates in cortex decreased following cis-flu infusions (*left*; *, $p < 0.05$). The magnitude of this decrease was significantly different from saline-related changes in mean firing rate (*right*; *, $p < 0.05$). D) Mean firing rates in GPe also decreased following cis-flu infusions (*left*; *, $p < 0.05$). The magnitude of this decrease did not appear to be much different from saline-related changes in average firing rate; however, there were not enough GPe cells recorded during saline injections to test this directly (*right).*

The decrease in cortical firing rates that we observed following cis-flu infusions (-81 +/- 5 %) was significantly different from changes in cortical firing rates following saline infusions (-2 +/- 36% change; $p < 0.05$). GPe firing rates also decreased following cis-flu infusions from 96 +/- 12 to 76 +/- 6 spk/sec ($p < 0.05$). There were not enough GPe cells recorded during saline injections to statistically compare cis-flu-related changes in GPe firing rates with saline-related changes; however, a similar trend was noticeably absent after saline infusions.

Intrastriatal DA receptor blockade also increased burstiness in cortical cells from 11 +/- 3 to 26 +/- 4 spikes in burst ($p < 0.05$; See Figure 13).

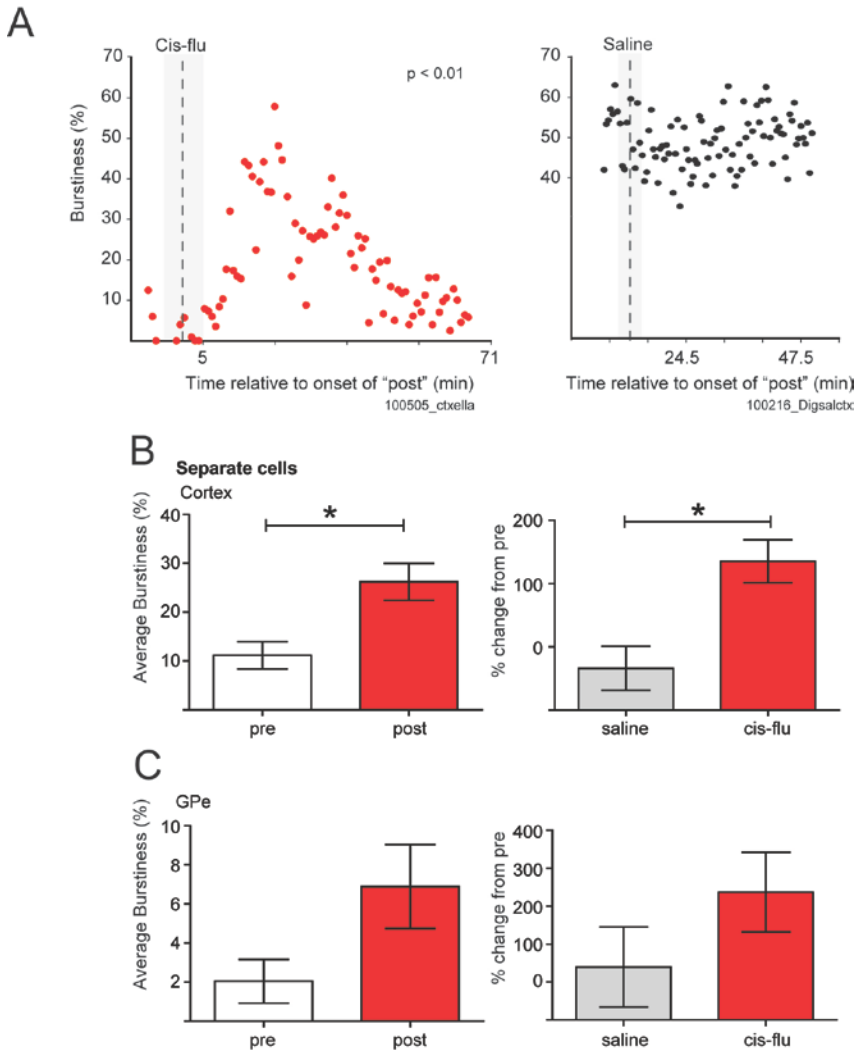

Figure 13: Changes in burstiness following injections

A) The mean resting burstiness values of one cortical cell (in 20-trial increments) throughout one cis-flu injection (*red*) is depicted on the left. The mean resting burstiness values of another cortical cell (in 20-trial increments) throughout a saline injection (*black*) is depicted on the right. B) Mean burstiness increased following cis-flu infusions in the cortex (*left*; *, $p < 0.05$). The magnitude of this increase was significantly greater than saline-related changes in burstiness (*right*). C) Mean burstiness also increased in GPe following cis-flu infusions, but this change was not significant (*left*). The magnitude of this increase was greater than saline-related changes in burstiness, but there were not enough GPe cells recorded during saline injections to make this comparison directly (*right*).

This increase in burstiness following cis-flu infusions (135 +/- 34%) was greater than saline-related changes in burstiness (-34 +/- 35%; $p < 0.05$). The burstiness of GPe cells also tended to increase after cis-flu infusions from 2 +/- 1% to 7 +/- 2% spikes in burst, but this change was not significant ($p > 0.05$). There were not enough GPe cells recorded during saline injections to compare cis-flu-related changes in GPe burstiness with saline-related changes; however, similar trends in burstiness were noticeably absent after saline infusions.

In summary, cis-flu infusions suppressed firing rates in cortex and GPe, while increasing burstiness in cortex. Burstiness also tended to increase in the GPe after cis-flu infusions, but this change was not significant.

3.3.2 Relationships between firing rates/burstiness and behavior

The behavioral relevance of changes in firing rate and burstiness was examined as well. Across all pre-injection periods, there was a slightly negative relationship between cortical average firing rates and contralateral RTs (Spearman rho = -0.13 +/- 09; mean +/- SEM; See Figure 14). Following cis-flu infusions, the average rho value significantly increased, but only to approximately zero (rho = 0.026 +/- .03; $p < 0.05$ Mann Whitney test). Therefore, changes in cortical firing rates became less related, or more variably related, to fluctuations in trial-by-trial RTs following cis-flu infusions. Thus, the relationship between cortical firing rates and the speed of contralateral RTs was altered by intrastriatal DA receptor blockade.

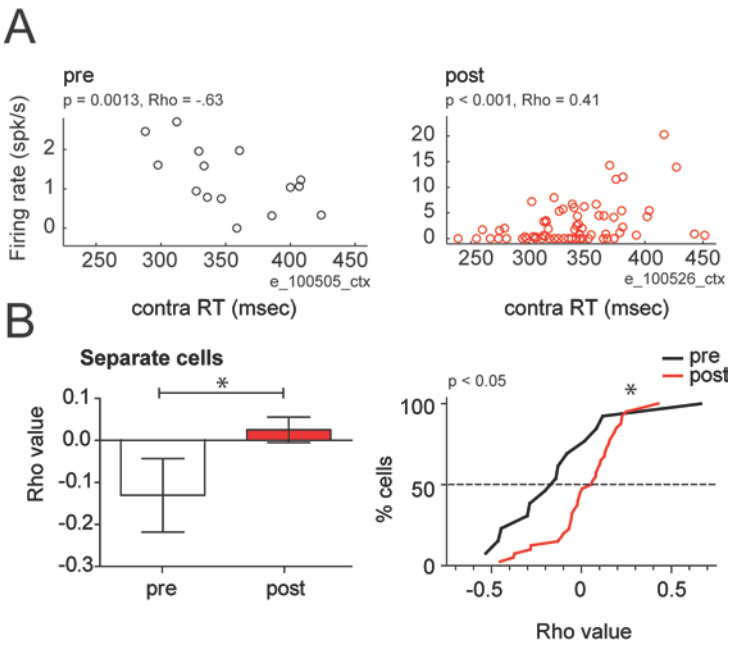

Figure 14: Changes in the relationship between cortical firing rates and contralateral reaction times

A) An example of a cell recorded pre-injection that exhibited a negative relationship between firing rate and contralateral RTs is depicted on the left (*black circles*). An example of a cell recorded post-injection that exhibited a positive relationship between average firing rate and contralateral RTs is depicted on the right (*red circles*). B) Before cis-flu infusions, there tended to be a negative relationship between cortical average firing rates and contralateral RTs. Following cis-flu infusions, the tendency for a negative relationship was reduced. More cells tended to exhibit no correlation or a positive correlation post-injection. The average rho value of correlations between cortical firing rates and contralateral RTs was negative before injections, but increased to approximately zero post-injection (*left; *, $p < 0.05$*). The cumulative distribution of rho values was also different pre- vs. post-injection (*right; *, $p < 0.05$*). In this cumulative distribution plot, note the tendency of more cells to: 1) exhibit a negative correlation between mean firing rates and contralateral RTs pre-injection and 2) exhibit no relationship or a positive correlation between mean firing rates and contralateral RTs post-injection.

There was a similar drug-related change in the relationship between contralateral RTs and cortical burstiness. Specifically, the average rho value increased from -0.2 +/- .07 to -0.04 +/-

0.03 following cis-flu infusions, but this change was not significant ($p > 0.05$, not pictured). There were no drug-related changes in the relationships between contralateral MTs or HTs and cortical firing rates or cortical burstiness. In the GPe, cis-flu infusions did not alter the relationships between contralateral RTs, MTs, or HTs and burstiness or average firing rates. Therefore, only the relationship between contralateral RTs and cortical average firing rates was altered by intrastriatal DA receptor blockade. The relationship between contralateral RTs and cortical burstiness was similarly affected, but this change did not reach significance. In sum, intrastriatal DA receptor blockade selectively disrupted the relationship between cortical activity and contralateral arm RTs.

3.3.3 Peri-event activity

Cis-flu infusions suppressed movement-related activity in cortical cells. Specifically, movement-related changes in cortical activity decreased from 12.2 +/- 6 spk/s to -0.7 +/- 3 spk/s ($p < 0.05$; Wilcoxon test; See Figure 15) following intrastriatal DA receptor blockade. There were no drug-related changes in peri-movement activity in the GPe.

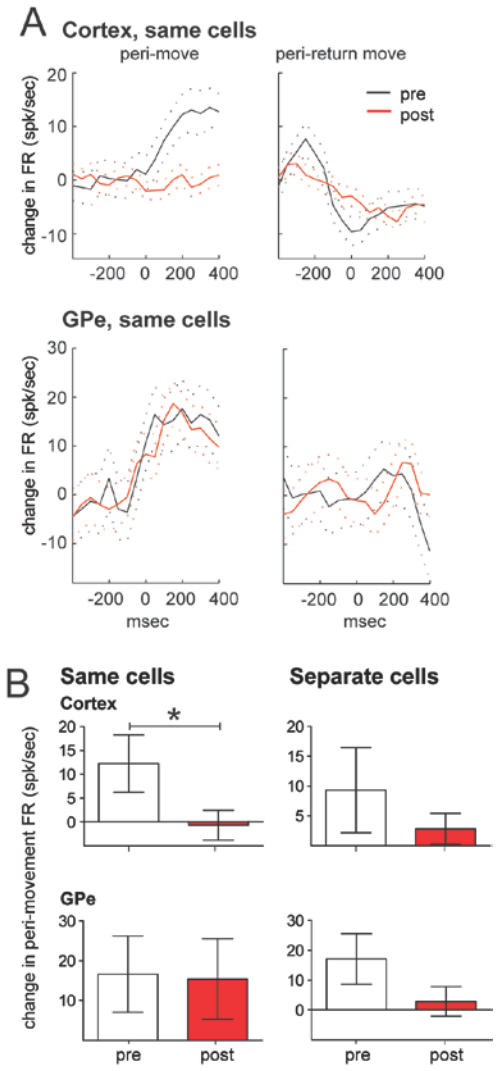

Figure 15: Peri-event activity

In the cortex, peri-movement activity of cells recorded both pre- and post- injection was suppressed following cis-flu infusions (A, *top left* and B, *top left*, *, $p < 0.05$). The peri-movement activity of cortical cells that were only recorded pre- injection tended to be higher than the peri-movement activity of cortical cells that were only recorded post-injection (B, *top right*); however, this change was not significant. Peri-return movement activity in the cortex was unaffected by cis-flu infusions (A, *top right*). In the GPe, cis-flu infusions did not affect peri-movement (A, *bottom left*) of cells recorded both pre- and post-injection (*B, bottom left*). The peri-movement activity of cells that were only recorded pre- injection tended to be higher than the peri-movement activity of cells that were only recorded post-injection (B, *bottom right*), but this change was not significant. Peri-return movement activity in the GPe was not affected by cis-flu infusions (A, *bottom right*). All of these

comparisons were performed using peri-movement activity during reaches with the arm contralateral to the hemisphere recorded.

3.3.4 LFP oscillatory activity

LFP signals were used to determine injection-related changes in oscillatory synchronous activity (See Figure 16).

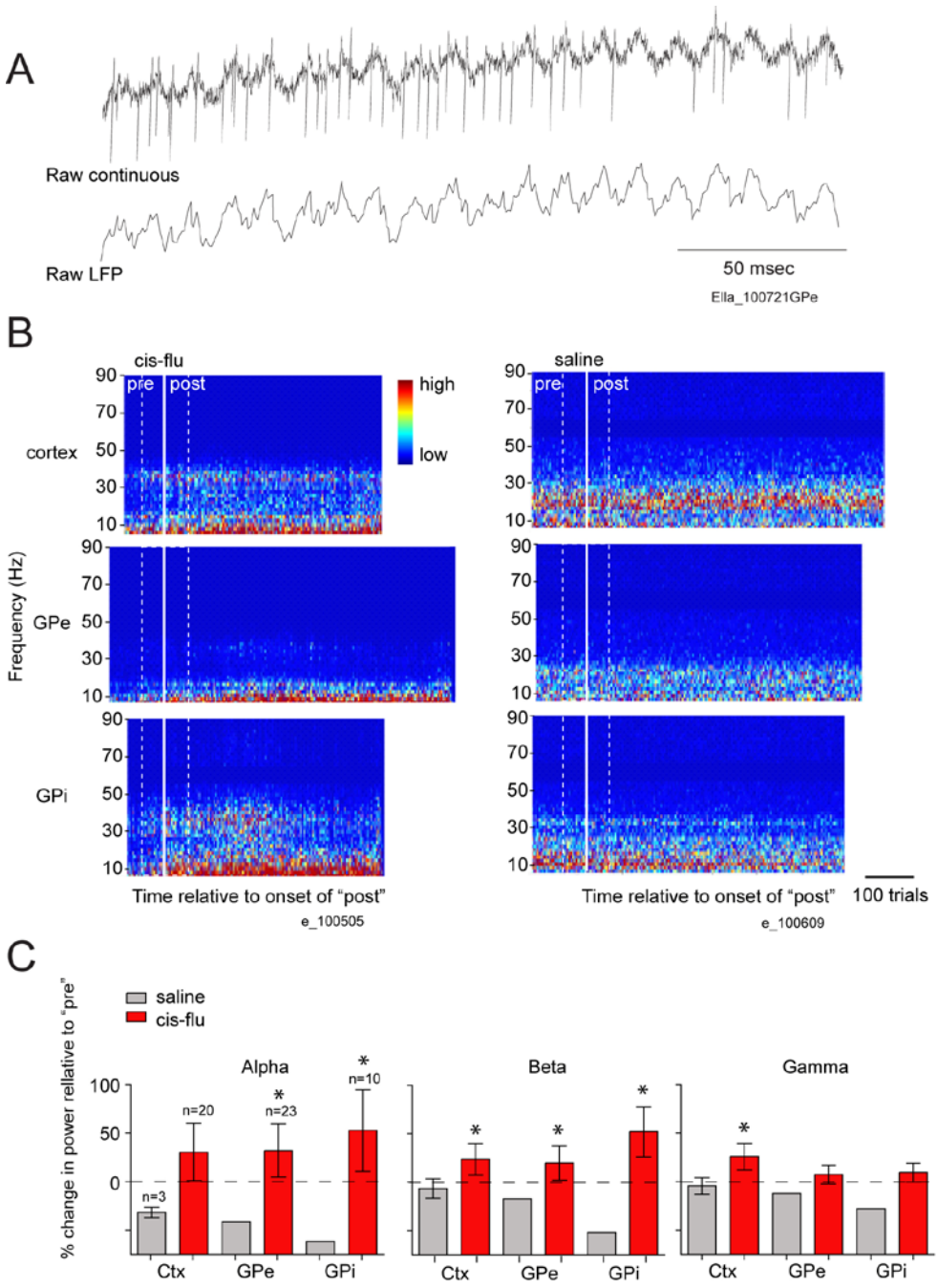

Figure 16: LFP activity

A) An example segment of LFP signal is depicted below the raw continuous data from which it was derived. B) Heatmaps were used to illustrate simultaneously recorded oscillatory activity in the cortex (*first row*), GPe (*second row*), and GPi (*third row*) throughout one cis-flu infusion (*left column*) and throughout one saline infusion (*right column*). The cis-flu infusion increased (*yellow*/*red*) the oscillatory activity across multiple frequency ranges in all three structures. In contrast, there was no obvious change in oscillatory activity

following the saline infusion. C) Cortical beta and gamma power increased following cis-flu infusions (*, $p < 0.05$). Alpha and beta power in the GPe and GPi also increased following cis-flu infusions (*, $p < 0.05$). Similar changes were not observed following saline infusions.

Following cis-flu infusions, relative β-and low gamma power increased in cortex (See Figure 16B for an example). In the GPe and GPi, α- and β-power also increased following cis-flu injections. Notably, there were no increases in α-, β-, or low gamma power in any structure following any of the saline infusions (See Figure 16B and 16C). Therefore, intrastriatal DA receptor blockade was sufficient to increase β-power in all three structures and increase low gamma and α- power in the BG and cortex, respectively.

3.3.5 Spike-triggered LFP activity

To determine whether drug-related changes in oscillatory activity were reflected in spiking activity, we quantified the temporal relationship between spiking activity and fluctuations in LFPs. Specifically, a spike-triggered LFP was computed for each cell with a good quality LFP signal on the same electrode (See Figure 17A). Twelve out of fifteen cortical cells, eighteen out of nineteen GPe cells, and three out of four GPi cells exhibited significant spike-triggered LFPs before cis-flu injections, indicating their spiking activity was time-locked, or synchronized with, fluctuations in LFPs. Chi-squared analyses revealed that striatal DA receptor blockade increased the proportion of cortical cells with spiking activity that was synchronized to fluctuations in LFPs ($p = 0.01$, $\chi^2 = 6.4$; See Table 3). This indicates that intrastriatal cis-flu injections increased synchronized activity in cortex.

Table 3: Fraction of cells with significant spike-triggered LFPs

	Pre-Injection	Post-Injection
Cortex	12/15 (80%)	30/30 (100%) *
GPe	18/19 (95%)	39/39 (100%)
GPi	3/4 (75%)	3/3 (100%)

This table summarizes the fraction (and percent) of cells with significant spike-triggered LFPs in the cortex, GPe, and GPi pre- vs. post-infusions of cis-flu. The number of cells that were significantly time-locked to LFP activity in the cortex significantly increased following cis-flu infusions (*, $p < 0.05$).

There was no drug-related change in the proportion of GPe or GPi cells with significant spike-triggered LFPs. In other words, intrastriatal injections did not alter synchronous activity in the GPe or GPi.

To determine whether synchronous activity became more oscillatory in nature, we generated a power spectrum of each spike-triggered LFP (See Figure 17A). These analyses revealed drug-related increases in β-power (from 7 +/- 1.5 to 15.5 +/- 4.1) and low gamma power (from 7.8 +/- 1.7 to 13.8 +/- 1.8; See Figure 17B) in the cortex. Only the increases in gamma power were significant, however ($p < 0.05$). This indicates that the spiking of cortical cells became more entrained to low gamma (and possibly β-) oscillatory activity following intrastriatal DA receptor blockade. Spike-triggered LFPs of GPe cells exhibited no drug-related changes in α-, β-, or low gamma power ($p > 0.05$; See Figure 17B).

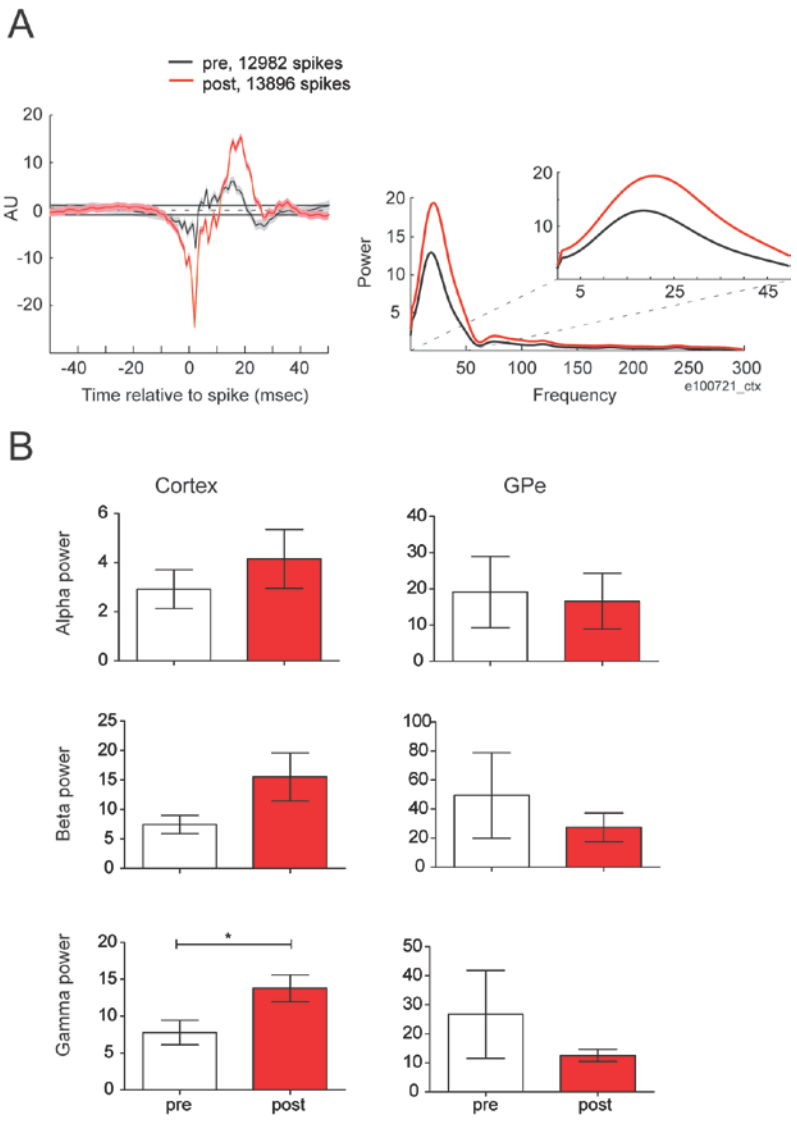

Figure 17: Spike-triggered LFPs

A) The peri-spike LFP activity of one example cell increased after an infusion of cis-flu (*left*). Computing the power spectra of these peri-spike LFPs revealed that the cis-flu infusion increased oscillatory power (*right*). B) The spiking of cortical cells became more entrained to low gamma (*, $p < 0.05$) and possibly *β-activity* (although $p > 0.05$) following intrastriatal DA receptor blockade (*left column*). Spike-triggered LFPs of GPe cells exhibited no drug-related changes in *α-*, *β-*, or low gamma power ($p > 0.05$; *right column*).

In sum, we found evidence that intrastriatal DA receptor blockade increased synchronous activity in the cortex. Furthermore, cortical spiking activity became more synchronous with low

gamma, and possibly β-activity following cis-flu infusions. No drug-related changes in synchrony were found in the GPe.

3.4 DISCUSSION

3.4.1 Suppression of cortical and GPe firing rates following intrastriatal DA receptor blockade

According to the rate model, striatal DA loss causes parkinsonian signs by setting off a cascade of firing rate changes throughout the BG nuclei that ultimately leads to a reduction in cortical firing rates (Albin et al., 1989; DeLong, 1990; Miller and DeLong, 1987; Wichmann and DeLong, 1996). Consistent with this hypothesis, previous studies have demonstrated that resting M1 firing rates are suppressed in parkinsonian macaques (Parr-Brownlie and Hyland, 2005; Pasquereau and Turner, 2010). These findings disagree with other studies reporting no change in cortical firing rates in the parkinsonian state (Doudet et al., 1990; Goldberg et al., 2002; Parr-Brownlie et al., 2007). The reason for this discrepancy is not clear, but could be attributable to differences in species or tasks employed. Furthermore, the suppression of cortical firing rates in parkinsonian macaques was recently found to occur selectively in pyramidal tract neurons, and not corticostriatal neurons (Pasquereau and Turner, 2010). It is therefore possible that studies reporting no changes in cortical firing rates in the parkinsonian state included a disproportionately large number of corticostriatal neurons.

The experimental approach employed here allowed us to specifically test whether striatal DA loss alone was sufficient to suppress cortical activity. Consistent with the rate model, we

found that intrastriatal DA receptor blockade suppressed cortical firing rates. In further agreement with the rate model, we also found evidence that striatal DA loss suppressed GPe firing rates. The suppression of GPe firing rates may have contributed to the suppression of cortical firing rates (as suggested by the rate model), thereby impairing the initiation and execution of movements. Unfortunately, the pathophysiologic relevance of aberrant BG activity (e.g., suppressed GPe activity) and disruption of cortical activity (e.g., suppressed firing rates) is difficult to tease apart. We found evidence, however, that striatal DA loss is sufficient to suppress firing rates in both the BG and cortex.

3.4.2 Intrastriatal DA receptor blockade increases burstiness

In addition to changes in firing rates, increased bursting occurs in both the cortex and BG of parkinsonian animals and in PD patients (Bergman et al., 1994; Boraud et al., 1996; Filion and Tremblay, 1991; Goldberg et al., 2002; Hutchison et al., 1997; Pasquereau and Turner). Consistent with these reports, we found increased burstiness in cortex, and a tendency for increased burstiness in the GPe following intrastriatal DA receptor blockade. Our inability to identify a significant increase in GPe burstiness could have been attributable to technical limitations. Due to the spatial arrangement of the microinjection and recording apparatus, it was impossible to record activity in the same region of the GPe during each injection. Therefore, changes in burstiness may have occurred in isolated territories of the GPe that were not sampled frequently enough here to yield overall significant increases in burstiness. Thus, increased burstiness in GPe may have been propagated to cortex, despite being insignificantly increased.

It is also important to consider other potential mechanisms whereby striatal DA loss increased cortical burstiness without inducing a significant increase in GPe burstiness. Increases

in cortical burstiness could have emerged due to increased burstiness in the direct pathway. Alternatively, increases in cortical burstiness following striatal DA receptor blockade may have been mediated by presynaptic DA receptors on corticostriatal neurons (Garcia-Munoz et al., 1991). Indeed, the intrastriatal administration of DA agonists was found to reduce the excitability of corticostriatal neurons. Therefore, it is possible that suppression of striatal DA might enhance the excitability of these neurons, increasing their bursting activity.

The pathophysiologic relevance of increased cortical bursty activity remains speculative. Increased bursting in the premotor and motor cortex could disrupt normal activity, muddling the clear generation of movement-related signals. Alternatively, increased bursting might reflect a misinterpretation of information coming from other circuits. The inability of different brain regions (such as thalamus and cortex) to communicate effectively after striatal DA loss could cause increased bursts of meaningless activity that are insufficient to initiate or execute movement. This could result in slowed initiation or execution of movement. Indeed, we also have evidence that cortical activity becomes less meaningful and somewhat less coupled to behavior following intrastriatal DA receptor blockade.

3.4.3 Intrastriatal DA receptor blockade alters the relationship between cortical FR and RTs

Movement-related cortical activity became less related to the speed of movement initiation following intrastriatal DA receptor blockade. Specifically, intrastriatal DA receptor blockade degraded the relationship between cortical firing rates and contralateral RTs. We observed a similar trend in the relationship between cortical burstiness and the speed of contralateral RTs as well, but this change was not significant. While small, these changes suggest that cortical

activity becomes less coupled to movement preparation following intrastriatal DA receptor blockade. It is possible that this decoupling of cortical activity and behavior manifested as the prolonged initiation of movements, or akinesia. Interestingly, M1 activity in parkinsonian macaques was also found to be characterized by bursts of synchronous discharge that are no longer related to movement (Goldberg et al., 2002).

It was quite surprising that we did not find any other drug-related changes in the relationship between cortical firing rates and HT or MT. This could be attributable to the fact that we were only looking at resting cortical firing rates and burstiness. It is less likely that cortical activity during the resting period would be related to the speed of HTs and MTs, as HTs and MTs occur much later in time. Finally, we did not observe any changes in the relationship between GPe firing rates or burstiness with RTs, HTs, or MTs. Again, this could be attributable to an incomplete sampling of the motor GPe. Alternatively, the decoupling of cortical activity from movement may have emerged without being propagated through the GPe, or may have been caused by a different type of aberrant BG output altogether. Importantly, this finding suggests that dysfunction of cortical activity following striatal DA loss may contribute to the development of parkinsonian signs.

3.4.4 Suppression of peri-movement activity in cortex following intrastriatal DA receptor blockade

Previous work from our lab has shown that the parkinsonian state is associated with a reduction in peri-movement activity in the M1 (Turner, 2007). A reduction in event-related activity was reported in the SMA of parkinsonian macaques during both the instruction and behavioral stages of a delayed motor task as well (Escola et al., 2003). Consistent with these

findings, we observed a suppression of movement-related activity in the cortex following intrastriatal DA receptor blockade.

These reductions in peri-movement activity could contribute to parkinsonian motor signs such as akinesia. If reductions in DA signaling in the BG attenuate activity throughout the BG-corticothalamic loops, peri-movement activity in the premotor cortex may never reach an arbitrary threshold of activity necessary to initiate movements (Lee and Assad, 2003). Similarly, peri-movement activity in the M1 could impede the execution of a motor program, representing a pathophysiologic substrate of bradykinesia (Escola et al., 2003). In sum, suppressed peri-movement activity in premotor cortex and M1 may represent pathophysiologic substrates for akinesia and/or bradykinesia.

Interestingly, a similar suppression of movement-related activity was not observed in the GPe following intrastriatal DA receptor blockade. While GPe cells recorded only pre-injection exhibited more peri-movement activity than GPe cells recorded only post- injection, this analysis is subject to a sampling bias, and may simply reflect a reduced tendency to isolate movement-related GPe cells during the post-injection period, rather than a suppression of GPe peri-movement activity. We may not have seen a change in peri-movement activity in the GPe because many of the cells may have been located outside the motor territory of the GPe. However, the strong peri-movement modulation of activity that we observed in GPe cells argues against this possibility. Thus, it remains unlikely that the suppression of peri-movement activity we observed in cortex is attributable to the suppression of peri-movement activity throughout the entire BG.

More likely, the suppression of cortical peri-movement activity is attributable to another factor, such as alterations in peri-movement activity that were isolated to the direct pathway.

Indeed, Leblois et. al (2007) have reported a pathologic increase in GPi task-related activity in parkinsonian macaques that could result in a suppression of peri-movement activity in cortex. They suggest that this increase in peri-movement GPi activity could be attributable to diminished inhibition through the direct pathway alone. In their earlier work, this group also found that disruptions in GPi peri-movement activity are associated with bradykinesia (Leblois et al., 2006). Based on these findings, they suggest that changes in peri-movement activity in the BG-cortical network may represent the most important pathophysiologic substrate of bradykinesia.

While the true pathophysiologic relevance of peri-movement activity changes therefore remains unclear, our results demonstrate that striatal DA loss is sufficient to suppress cortical peri-movement activity. This lends further support to the idea that alterations in peri-movement activity in the BG-cortical network may represent an important pathophysiologic substrate for parkinsonian signs (Leblois et al., 2006). Finally, this suggests that striatal DA loss may cause parkinsonian signs by causing cortical dysfunction.

3.4.5 Increased oscillatory activity in cortex, GPe, and GPi following intrastriatal DA receptor blockade.

In the parkinsonian state, cortical and BG activity is known to become more oscillatory in nature. Such PD-related increases in oscillatory activity are thought to be frequency-specific. Increases in β-oscillations, in particular, are a well-established finding in the BG in parkinsonian patients off medications (Brown et al., 2001; Levy et al., 2002a; Silberstein et al., 2003). β-activity can be modulated by dopaminergic medication (Kuhn et al., 2006; Levy et al., 2002a) and DBS (Kuhn et al., 2008) and is thought to be related to the severity of specific parkinsonian signs (Kuhn et al., 2008; Kuhn et al., 2006). While β-activity can be recorded locally, it is likely

shared across BG-cortical circuits. Indeed, coherent β-oscillations have been identified in the STN and cortex of PD patients (Marsden et al., 2001). Taken together, these results suggest that increased β-activity throughout the BG-cortical loops is associated with the pathophysiology of PD.

Recent evidence, however, casts doubt on the idea that β-oscillations are related to the signs of PD. Specifically, β-oscillations in macaques were reported to emerge long after the development of parkinsonian signs (Leblois et al., 2007), suggesting they do not contribute to akinesia and bradykinesia, two early signs of PD. Therefore, the specific cause and pathophysiologic relevance of β-oscillations is unclear. Here, we tested whether striatal DA receptor blockade was sufficient to acutely increase β-activity in the cortex and BG. As predicted, we observed a significant increase in β-activity in the cortex, GPe, and GPi following cis-flu infusions. Importantly, these changes were found to emerge within a typical experimental session (< 1.5 hours).

The mechanism whereby increased β-activity could cause parkinsonian signs remains unclear. β-activity may suppress movement by interfering with oscillatory activity in other frequencies that are necessary for movement (Brown and Williams, 2005). Alternatively, overexpression of β-activity might prevent movement by promoting the existing, or resting motor state (Gilbertson et al., 2005). β-activity might also alter motor behavior by influencing neuronal activity. Indeed, cortical activity was found to be depressed during periods of 20-40 Hz cortical oscillatory activity (Murthy and Fetz, 1996). Furthermore, the tendency of modulations in cortical firing rates to occur is enhanced as oscillatory activity decreases in M1 (Donoghue et al., 1998). In accordance with these findings, we observed an increase in cortical oscillatory activity along with a suppression of cortical firing rates and reduced peri-movement modulations

of firing rates. These changes may actually be inter-related and could explain how the suppression of peri-movement activity could occur in the cortex without similar reductions in GPe peri-movement activity.

In addition to changes in β-activity, α- activity also increased in the GPe and the GPi following intrastriatal DA receptor blockade. This is consistent with previous studies demonstrating that increased α- activity in the BG is associated with the parkinsonian state (Bergman et al., 1994; Kuhn et al., 2004; Priori et al., 2004; Ray et al., 2008). Despite increases in α- activity in the BG, cortical α- activity in the cortex was not increased by cis-flu infusions. Suppressing striatal DA activity may have therefore enhanced the entrainment of GPe and GPi to ongoing, lower frequency cortical activity, without increasing the power of cortical α- activity directly. Indeed, there is evidence that the BG's ability to process cortical oscillatory activity is disrupted in the parkinsonian state (Williams et al., 2002). This supports the idea that striatal DA loss may cause parkinsonian signs by causing dysfunction of the normal BG.

Surprisingly, we did observe a drug-related increase in cortical low gamma activity. The implications of this finding, however, remain unclear. Low gamma activity is thought to be related to sensory binding (Engel et al., 2001), attention selection (Fries et al., 2001), and memory (Fuchs et al., 2007; Howard et al., 2003). Therefore, our findings may reflect an increased level of attention that was required to perform the task following intrastriatal DA receptor blockade. Indeed, on some trials the animals were able to overcome behavioral deficits incurred by the injections (notice the variability of HTs in Figure 7A). This may have been due to periodic increases in the animals' conscious effort to overcome the deficit, and may have been reflected physiologically as an increase in cortical low gamma activity.

Interestingly, inhibitory interneurons have been implicated in the generation of low gamma activity (Dupret et al., 2008). Therefore, an alternative explanation for increased cortical gamma activity is that striatal DA loss altered the activity of inhibitory interneurons, promoting increased low gamma oscillatory activity. Indeed, there are reports of reduced intracortical inhibition in the M1 of PD patients that are thought to be due to alterations in cortical interneuron activity (Hanajima and Ugawa, 2000; Ridding et al., 1995); however, this finding has not been consistently replicated (Berardelli et al., 1996b).

Taken together, our results are consistent with previous findings that oscillatory activity in the BG and cortex, particularly in the β-frequency, is enhanced by striatal DA loss. Fogelson et al (2006) postulated that oscillations in separate frequencies serve to segregate information traveling through separate functional loops. Therefore, our finding that specific frequencies of oscillatory activity are particularly enhanced following intrastriatal DA receptor blockade could reflect a loss of integrity between separate functional loops.

3.4.6 Increased synchronous activity in the cortex following intrastriatal DA receptor blockade.

We expected that the changes in oscillatory activity that we detected using LFP signals would also be reflected in spiking activity. Indeed, STN cell spiking is synchronized with β-activity in the STN of PD patients (Weinberger et al., 2006). In many cells, we found that spiking was directly related to fluctuations in LFP activity both before and after intrastriatal DA receptor blockade. Interestingly, a greater number of cortical neurons became entrained to fluctuations in the LFP signal after intrastriatal DA receptor blockade. This reflects an increase in cortical population synchrony. The mechanism whereby changes in cortical synchrony could

induce parkinsonian signs remains unclear; however, some propose that increased synchrony limits the coding abilities of neuronal activity that may be necessary for movement (Brown and Williams, 2005).

Interestingly, we found evidence that the increased synchronous activity in cortex following striatal DA receptor blockade was oscillatory in nature. Cortical spiking activity became more entrained to low gamma oscillatory activity following injections. The implications of this are unclear. It could reflect an increased tendency for spiking activity to become synchronized to the activity of inhibitory interneurons generating gamma oscillatory activity. Cortical spiking activity also became more entrained to β-activity, but this increase was not significant. In general, we found evidence that cortical neuron populations become more synchronized and oscillatory in the absence of striatal DA.

Somewhat surprisingly, we found no evidence for an increase in synchronous activity in the GPe. Specifically, we did not observe a significant increase in the number of GPe neurons that exhibited spiking activity entrained to fluctuations in LFP activity. This does not suggest that the aforementioned increases in GPe oscillatory activity were not reflected in spiking activity. Recall that a large proportion of GPe cells were synchronized with fluctuations in LFPs both before and after injections. Therefore, it is likely that spiking activity in the GPe reflected oscillatory activity equally both before and after injections. In sum, these results simply indicate that there was no change in synchronous activity in the GPe.

How could striatal DA loss increase synchrony in cortex without also affecting GPe activity? It is possible that inadequate sampling of the motor territory of the GPe impeded our ability to detect increased synchrony. Instead, a different type of abnormality in the BG (such as increased oscillatory activity) may have caused increased synchronous activity in cortex.

Alternatively, synchronous activity may have been directly propagated to the cortex through the direct pathway alone, without any changes in synchronous activity within the GPe. While we found no change in synchronous activity in the GPi, there are likely too few GPi cells to exclude this possibility entirely. It is also possible that pre-synaptic modulation of cortical activity following striatal DA loss may have caused changes in synchronous activity, without direct involvement from the BG (Garcia-Munoz et al., 1991). As previously mentioned, fluctuations in striatal DA levels were found to alter excitability of corticostriatal neurons. Resulting enhancements of cortical excitability may have increased the likelihood for synchronous firing in cortex.

3.5 CONCLUSIONS

The development of parkinsonian signs may be attributable to specific changes in neuronal activity in the BG and/or cortex. The specific cause and pathophysiologic relevance of these changes remains unclear. Here, we found that intrastriatal DA receptor blockade dramatically affected cortical activity in the following ways: 1) resting firing rates were suppressed; 2) burstiness was increased; 3) resting activity became less coupled to movement initiation 4) peri-movement activity was reduced; 5) oscillatory activity was increased; and 6) synchronous activity was increased. In the GPe, resting firing rates were suppressed, oscillatory activity was increased, and a tendency for increased bursting was observed. These findings are consistent with the rate model and with reported changes in cortical and BG activity in parkinsonian animals and PD patients. These findings also demonstrate that striatal DA loss is sufficient to induce specific changes in cortical and BG activity patterns that are associated with the

parkinsonian state. Possibly, separate changes in BG and cortical activity following striatal DA loss differently contribute to different aspects of parkinsonian signs. While the pathophysiologic roles of specific changes in neuronal activity patterns cannot be delineated here, this work highlights the importance of cortical dysfunction in the development of parkinsonian signs. Future work is necessary to delineate the clinical relevance of specific cortical and BG activity changes in PD.

4.0 SUMMARY AND CONCLUSIONS

4.1 INTRASTRIATAL DA RECEPTOR BLOCKADE IS SUFFICIENT TO INDUCE SPECIFIC PARKINSONIAN SIGNS

The development of parkinsonian signs is thought to be caused by striatal DA loss. Evidence for this hypothesis is ubiquitous. The striatum is almost completely devoid of DA in PD patients (Forno, 1996; Kish et al., 1988). Furthermore, neurotoxins that deplete striatal DA levels induce parkinsonian-like signs in animals and humans (for review, see Emborg, 2007). Finally, DA replacement therapy (DRT) ameliorates parkinsonian signs in both animals and humans. While these findings are consistent with the idea that striatal DA loss causes the signs of PD, they do not preclude the possibility that other pathologic processes may also contribute to the development of parkinsonian signs. Both PD and animal models of PD are associated with various other pathologic changes such as extrastriatal DA loss, depletion of other neurotransmitters, and cell death in the SNpc (see *Animal Models of PD* in 1.3 Pathophysiology of PD). Given our current knowledge, the relative contribution of each of these pathologic processes to the development of parkinsonian signs is impossible to tease apart. Furthermore, the widespread effect of DRT makes it difficult to delineate the importance of restoring striatal or extrastriatal DA in ameliorating parkinsonian signs. Thus, it remains unclear whether striatal

DA loss is sufficient to cause parkinsonian signs. Here, we tested the fundamental, long-accepted hypothesis that striatal DA loss is sufficient to induce specific parkinsonian signs.

We infused D1- and D2- receptor antagonists directly into the putamen while macaques performed a bimanual reaching task to test this hypothesis. We found that 1) akinetic and bradykinetic effects could be induced independently following separate infusions; 2) intrastriatal DA receptor blockade induced primarily akinetic rather than bradykinetic effects; and 3) the initiation of self-generated movements was more impaired by intrastriatal DA receptor blockade than the initiation or execution of externally-cued movements.

The finding that akinetic and bradykinetic effects could be induced independently following separate infusions suggests that bradykinesia and akinesia represent distinct parkinsonian signs that likely have different pathophysiologic substrates. This questions the traditional perspective that akinesia is a more severe form of bradykinesia. The distinction between akinesia and bradykinesia is an important consideration in developing individualized therapies for patients with different symptomatic profiles.

The fact that bradykinetic effects were more rarely induced and smaller in magnitude than akinetic effects was surprising because DA loss in the motor area of the putamen has long been thought to cause movement slowing in PD patients (Kish et al., 1988). In contrast, we found that cis-flu injections performed directly into the motor arm area of the putamen predominantly slowed the initiation of movements rather than the execution of movements. It is therefore likely that striatal DA loss may contribute more to the akinetic signs of parkinsonism, while bradykinetic signs may be largely attributable to other pathologic processes such as cortical or pallidal DA loss. Alternatively, more extensive or long-term striatal DA loss might have induced stronger bradykinetic effects. Finally, DA loss in multiple striatal and extrastriatal

sites might have been necessary for severe bradykinesia to emerge. More studies are warranted to determine the pathophysiologic substrate of bradykinesia. Here, we demonstrate that acute striatal DA loss likely represents one pathophysiologic substrate of akinesia, primarily affecting the initiation of self-generated movements.

Interestingly, we were able to slow the initiation of self-generated and externally-cued movements independently. The initiation of self-generated movements was more severely affected than the initiation of externally-cued movements. In PD patients, self-initiated movements are more impaired than externally-cued movements (Flowers, 1976; Morris et al., 1996; Oliveira et al., 1997). External cues are frequently used to help PD patients initiate self-generated movements. Therefore, the form of akinesia we observed following intrastriatal DA receptor blockade reflects an akinetic state that is paralleled in PD patients.

The drug-related slowing that we observed during the initiation of self-generated movements was quite severe at times. However, the degree of slowing during the initiation of self-generated movements was not related to the degree of slowing during the initiation and execution of externally-cued movements. This supports the idea that the slowed initiation of self-generated movements is attributable to a separate pathophysiologic mechanism than slowed initiation and execution of externally-cued movements. Alternatively, externally-cued movements may have been less affected than self-generated movements because other brain regions were better able to compensate for disruptions in externally-cued movements. Future work is necessary to determine why externally-cued movements are less affected by acute striatal DA loss than self-initiated movements. Insight from such studies could elucidate potential compensatory mechanisms that are exploited during the generation of externally-cued

movements. Such findings could lead to new therapeutic approaches that could improve the initiation of self-generated movements, both in PD and other disorders.

In summary, we found evidence that different parkinsonian signs have distinct pathophysiologic substrates. We also discovered that acute striatal DA receptor blockade is sufficient to induce severe akinesia, one cardinal sign of parkinsonism. Interestingly, akinetic behavior induced by intrastriatal DA receptor blockade resembled akinesia described in PD patients, in that the initiation of self-generated movements was more severely affected than the initiation of externally-cued movements. We also found evidence that such akinetic signs may be caused, at least in part, by acute striatal DA loss, while bradykinetic signs may be attributable to a separate pathophysiologic substrate. These findings provide information that could be helpful in developing more individualized therapies for patients with distinct symptomatic profiles. Furthermore, this work highlights the importance of future studies exploring the potential pathophysiologic substrate of bradykinesia and determine the specific behavioral effects of extrastriatal DA loss.

4.2 INTRASTRIATAL DA RECEPTOR BLOCKADE IS SUFFICIENT TO INDUCE ABNORMAL NEURONAL ACTIVTY PATTERNS IN CORTEX AND GPE

Two overarching hypotheses could explain how striatal DA loss may induce parkinsonian signs: 1) a degradation of a normal function of the BG; or 2) dysfunction of BG-recipient cortical or brainstem regions. These hypotheses are difficult to tease apart, however, without knowing how striatal DA loss affects neuronal activity in the cortex and BG. Here, we sought to determine whether intrastriatal DA receptor blockade was sufficient to induce abnormal neuronal

activity patterns in the cortex and BG that are known to be associated with the parkinsonian state. The effects of intrastriatal DA receptor blockade that we observed on neuronal activity measures in the cortex and GPe are summarized in Table 4.

Table 4: The effects of intrastriatal DA receptor blockade on neuronal activity

This table summarizes the effects of acute intrastriatal DA receptor blockade on cortical and GPe activity. Up and down arrows indicate increases and decreases, respectively. Empty set indicates no change.

	Cortex	GPe
Resting firing rate	↓	↓
Burstiness	↑	∅
Relationship with behavior	↓	∅
Peri-movement activity	↓	∅
Synchronous activity	↑	∅
Oscillatory activity	↑	↑

The most prominent changes in neuronal activity that we found following intrastriatal DA receptor blockade were found in cortex. Consistent with our predictions, resting and peri-movement cortical firing rates were suppressed following intrastriatal DA receptor blockade. The resulting reduction in peri-movement activity superimposed on a lower background firing rate suggests that cortical cells were underactive and less responsive following intrastriatal DA receptor blockade. Such changes in the premotor and motor cortex may have manifested behaviorally as slowed movement initiation and execution, respectively.

We also observed a tendency for changes in cortical firing rates to become less related to the speed of contralateral RTs following cis-flu injections. While the magnitude of this change was small, it suggests that cortical activity might become less coupled to movement preparation in the absence of striatal DA. Indeed, cortical activity in parkinsonian macaques is characterized

by increased bursts of activity without corresponding behavioral responses (Goldberg et al., 2002). Similarly, we found that cortical activity became more bursty and less coupled to behavior in the absence of striatal DA. This represents another potential mechanism whereby striatal DA loss might have slowed movement initiation or execution, depending on the specific cortical area affected.

Striatal DA loss also increased oscillatory activity in cortex. Specifically, we found that low gamma and β-activity became more powerful in the cortex following intrastriatal DA receptor blockade. Since β-activity is known to be akinetic in nature, the enhancement of β-activity just prior to movement initiation could indicate another potential mechanism whereby striatal DA loss impaired movement initiation. Importantly, we found evidence that the drug-related changes in oscillatory activity we observed were likely reflected in spiking activity.

Synchronous activity in the cortex was also enhanced following intrastriatal DA receptor blockade. Increased cortical synchrony could play a role in the development of parkinsonian motor signs in various ways. The entrainment of large cortical cell populations to more global, synchronous activity could impair the selective activation of distinct cortical neuronal populations necessary for movement initiation (Pessiglione et al., 2005). This loss of information specificity could then muddle the generation of clear movement commands, resulting in bradykinesia and/or akinesia. A loss of selective neural responsiveness might also impair the ability to activate only specific muscle groups, resulting in simultaneous co-activation of many muscles, or rigidity. Each of these possibilities represents a potential mechanism whereby intrastriatal DA receptor blockade may have slowed the initiation and execution of movements.

In addition to these effects on cortical activity, acute striatal DA receptor blockade reduced resting firing rates and tended to increase bursting in the GPe. Oscillatory β-activity was also increased in both the GPe and GPi. It is therefore possible that decreases in cortical firing rate and increases in cortical burstiness and β-power could have been directly communicated to the cortex by the BG. It remains unclear, however, whether the presence of these abnormalities in cortex or the BG is more pathophysiologically relevant.

Importantly, many of the changes we observed in cortex were not found in the GPe. For example, striatal DA receptor blockade reduced peri-movement activity, increased synchrony, and altered the relationship between cortical activity and behavior in the cortex, but not the GPe. How could striatal DA loss induce changes in cortical activity without affecting GPe activity? Some possible explanations for this finding include: 1) presynaptic modulation of cortical activity (Garcia-Munoz et al., 1991); 2) modulation of cortical activity by the direct pathway only (Leblois et al., 2007); 3) an unidentified form of aberrant output from the BG that indirectly caused these changes in cortical activity; 4) disruption of information transmission from the BG to the thalamus, or from the thalamus to the cortex; or 5) insufficient sampling of the motor GPe which may have impeded our ability to detect some drug-related changes in GPe activity.

In general, we found that most drug-induced changes in neuronal activity occurred in cortex. Furthermore, we discovered that striatal DA loss specifically altered the relationship between cortical activity changes and behavior. These findings support the idea that striatal DA loss induces parkinsonian signs by causing cortical dysfunction, rather than by simply degrading normal functions of the BG. Importantly, however, alterations in cortical activity following striatal DA loss are likely attributable to aberrant BG output. Indeed, GPi lesions are known to ameliorate signs of PD (Coban et al., 2009; de Bie et al., 1999; Vitek et al., 1998).

It is also possible, however, that striatal DA loss can alter cortical activity directly via pre-synaptic mechanisms (Garcia-Munoz et al., 1991). Furthermore, there is evidence that alterations in some cortical activity patterns, such as oscillations and modulations in neuronal activity, can precipitate changes in one another (Donoghue et al., 1998; Murthy and Fetz, 1996). This could generate a cyclic development of aberrant cortical activity patterns that could contribute to parkinsonian signs. Future studies are necessary to determine how striatal DA loss can directly influence cortical activity. This may help identify an ideal target for therapeutic intervention.

The fact that striatal DA loss did not induce many of the abnormal GPe neuronal activity patterns that are known to be associated with the parkinsonian state may suggest that striatal DA loss is not sufficient to induce all abnormalities in neuronal activity that are associated with the parkinsonian GPe. These abnormal activity patterns may be induced by other pathologic processes associated with the PD, such as extrastriatal DA loss (for review, see Rommelfanger and Wichmann, 2010), or may simply require more extensive or long-term striatal DA depletion. Future studies are necessary to test these hypotheses. Relating such findings to behavioral changes could provide tremendous insight into the cause and pathophysiologic relevance of specific neuronal activity patterns in the BG.

4.3 CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, we discovered that striatal DA loss is sufficient to induce specific parkinsonian signs. Specifically, acute intrastriatal DA receptor blockade induced akinesia, affecting primarily the initiation of self-generated movements. Furthermore, we found that specific

parkinsonian signs likely have separate pathophysiologic substrates which should be considered in the development of more individualized therapies. These findings also suggest that restoring striatal DA levels may not be not sufficient to ameliorate all parkinsonian signs, as acute striatal DA receptor blockade was unable to induce all parkinsonian signs. Future complementary studies are necessary to determine whether restoring DA in striatal subregions is sufficient to ameliorate specific parkinsonian signs.

We also discovered that acute striatal DA loss is sufficient to induce abnormal activity patterns in cortex and BG that are associated with the parkinsonian state. Specifically, intrastriatal DA receptor blockade reduced resting and peri-movement activity and increased burstiness, oscillatory activity, and synchrony in the cortex. Suppression of striatal DA activity also decoupled cortical activity from movement. In the GPe, resting firing rates were reduced and burstiness tended to increase following intrastriatal injections of cis-flu. Finally, striatal DA loss increased oscillatory activity in the GPe and GPi. Taken together, these findings suggest that acute striatal DA loss primarily affected cortical activity, frequently without similar changes in the BG. This highlights the potential importance of cortical activity changes in the development of parkinsonian signs, and suggests that the development of parkinsonian signs may be attributable to cortical dysfunction.

Future work is necessary to determine the pathophysiologic substrate for parkinsonian signs, such as rigidity and bradykinesia that were either minimally elicited or not elicited by striatal DA receptor blockade. Similarly, we need to determine the pathophysiologic substrate and clinical relevance of abnormal neuronal activity patterns in the BG that were not induced here, such as increased synchrony and burstiness in the BG. Finally, the contribution of other pathologic processes that occur in the parkinsonian state, such as extrastriatal DA loss, to the

development of specific parkinsonian signs should be determined. Information from these studies could help to delineate the pathophysiologic substrates for specific parkinsonian signs. Furthermore, this information could elucidate the cause and clinical relevance of specific abnormal neuronal activity patterns associated with the parkinsonian state. Equipped with this knowledge, we can develop more individualized and effective therapeutic options for PD patients in the future.

APPENDIX A

BURSTS AND OSCILLATIONS AS INDEPENDENT PROPERTIES OF NEURAL ACTIVITY IN THE PARKINSONIAN GLOBUS PALLIDUS INTERNUS

A.1 INTRODUCTION

Please note that Appendix A was previously published as (Chan et al., 2011).

Parkinson's disease (PD) is a chronic neurologic disorder with cardinal motor signs of muscle rigidity, tremor, slowness of movement (bradykinesia), and/or paucity of movement (akinesia). The fact that lesions placed in the globus pallidus internus (GPi) significantly ameliorate most of these signs indicates that the GPi plays an important role in the pathophysiology of parkinsonian motor signs (Coban et al., 2009; de Bie et al., 1999; Vitek et al., 2003). In animal models of parkinsonism (Filion and Tremblay, 1991; Miller and DeLong, 1987; Raz et al., 2000), and in idiopathic PD (Dogali et al., 1994; Hutchison et al., 1994; Lozano et al., 1996; Vitek et al., 1998), GPi neurons exhibit a constellation of abnormalities in spiking activity including elevated firing rates and altered firing patterns.

Although classical models of PD pathophysiology focus on altered GPi firing rates, growing evidence suggests that an increased prevalence of burst discharges (Kaneoke and Vitek, 1996) may be more important in the pathophysiology of PD (Bergman et al., 1994; Boraud et

al., 1996; Boraud et al., 1998; Boraud et al., 2000; Filion and Tremblay, 1991; Hutchison et al., 1997; Starr et al., 2005; Wichmann and Soares, 2006). The mechanistic underpinnings of elevated bursting activity and its true clinical significance remain unclear, however. For example, medical and surgical therapies that reduce PD motor signs do not consistently reduce bursting activity in the BG (Chen et al., 2001; Hahn et al., 2008; Levy et al., 2001; McCairn and Turner).

In addition to increased burst discharges, oscillatory firing (OF; abnormal rhythmic modulations in firing rate) in the α- (8-13-Hz) and β-(13-30-Hz) frequency ranges is a common characteristic of BG activity in both PD patients and animal models of PD (Gatev et al., 2006; Levy et al., 2002bb; Rivlin-Etzion et al., 2006aa; Starr et al., 2005; Weinberger et al., 2006). Treatments that ameliorate PD signs [e.g., dopamine replacement therapy (DRT)] also reduce α- and β-frequency oscillations in spiking (Heimer et al., 2006; Levy et al., 2001) and local field potential (LFP) activity (Brown et al., 2001) in the GPi. Other anti-parkinsonian therapies, such as Deep Brain Stimulation (DBS) are also reported to decrease α- and β-frequency oscillations in the GPi (McCairn and Turner). However, not all studies support these findings (Foffani et al., 2006). Thus, the mechanisms and clinical significance of oscillatory firing also remain a topic of debate (Degos et al., 2009; Leblois et al., 2007; Mallet et al., 2008).

While oscillations and bursts are each consistent features of the parkinsonian BG, it remains unclear whether the two occur independently or are closely linked phenomena. The oscillations in neuronal firing rate associated with parkinsonism are often described as periodic bursts of neural activity, which has led to the frequent assumption that oscillations and bursts are closely linked, co-occurring phenomena (Rubin and Terman, 2004; Terman et al., 2002). For example, Galvan and Wichmann suggested that oscillations may be caused by rebound bursting

within BG loops (Galvan and Wichmann, 2008). In contrast, based on theoretical considerations, Kaneoke and Vitek proposed that oscillations and bursts may represent two distinct processes. Existing evidence suggesting they may be separate phenomena includes the observation that not all OF is bursty (Wichmann and Soares, 2006), and the fact that bursts and oscillations are not affected in a similar manner by pharmacologic and surgical therapies (see above). Here, we determined the degree to which OF and bursty activity in the GPi of PD patients are related to each other and to the severity of parkinsonian signs. An improved understanding of the relationship between bursts and oscillations will facilitate the analysis of pathophysiologic relationships between types of abnormal GPi activity patterns and specific parkinsonian signs.

A.2 METHODS

A.2.1 Patient Population

Single unit recordings in the GPi were obtained from patients with PD undergoing microelectrode-guided stereotactic surgery for the placement of GPi DBS electrodes. All patients were responsive to levodopa (3,4-dihydroxy-L-phenylalanine) and had developed levodopa-induced dyskinesias or motor fluctuations. The severity of disease was assessed prior to surgery according to 27 sections of the Unified Parkinson's Disease Rating Scale (UPDRS). The UPDRS scores were assessed by different neurologists approximately one month prior to surgery, both off and on DRT. The responsiveness to DRT for each patient was quantified as the percentage improvement in his or her total UPDRS score following DRT. Scores were not available for 3 of

the 14 patients. Anti-parkinsonian medications were withheld for at least 12-hr before the surgery and all PD patients displayed overt parkinsonian symptoms without dyskinesias during the procedure. PD patients with severe off-period dystonia were excluded from the study. All subjects gave informed consent according to a protocol approved by the University of California San Francisco Institutional Review Board. All work was carried out in accordance with the Code of Ethics of the World Medical Association.

A.2.2 Surgical procedures and data collection

The methods used for microelectrode-guided stereotactic implantation of DBS electrodes in the GPi were similar to those described previously (Starr, 2002). Single-unit recordings were obtained using glass-coated platinum/iridium microelectrodes with impedance 0.4–1.0-MΩ (Microprobe, Gaithersburg, MD, or FHC, Inc., Bowdoin, ME). Signals were bandpass filtered (300–5000-Hz), amplified, played on an audio monitor, displayed on an oscilloscope, and digitized (20-kHz sampling rate) using the Guideline System 3000 or 4000 (FHC, Inc.). Microelectrodes were advanced into the brain using a motorized microdrive (FHC, Inc.). In a typical surgical case, one to two microelectrode penetrations separated by 2–3-mm were made serially through the GPi on each side. The GPe and GPi were distinguished by recording a 1-2 mm interval of electrical silence corresponding to the white matter laminae between the GPe and GPi. Cells were recorded at approximately every 300–800-μm along each trajectory through the GPi. Spontaneous neuronal activity of well-isolated cells was collected for 37.7-sec on average (SD = 18-sec).

All patients were sedated with propofol for the initial surgical incision and skull opening. Propofol was stopped at least 30-min prior to neuronal recording, which is sufficient time to

wash out its known effect on single unit discharge (Raz et. al, 2008). All patients were awake and alert, and were asked to remain as still as possible with eyes open during periods of neuronal recording.

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A.2.3 Data Analysis

Digitized spike trains were imported into off-line spike sorting software (Plexon Inc., Dallas TX) for discrimination of single unit action potentials by cluster-cutting in principal components space. This software generated a record of the time of occurrence (reduced to millisecond accuracy) for each action potential waveform detected. The spike times were used to calculate discharge rate, bursting, and oscillatory activity (see following text). Analyses were performed in the Matlab computing environment (The Mathworks, Natick, Massachusetts). Neuronal data were included in this study only if action potentials could be discriminated with a high degree of certainty as indicated by the presence of a clear refractory period in the inter-spike interval (ISI) histogram (>3-msec). Neurons were excluded if the size of their action potentials varied considerably in unison with the cardiac cycle. Figure 18A illustrates the single unit isolation that was typical for the recordings used in this study.

A.2.4 Burst Firing

Bursts were detected using the Poisson Surprise Method (Legendy and Salcman, 1985; Wichmann et al., 1999). Each period of increased neuronal discharge was assigned a surprise value (S) that quantified the likelihood that this period of activity was a burst (i.e., a discrete period of elevated firing rate), rather than part of the neuron's ongoing stochastic firing pattern. The surprise value (S) can be represented as:

$$S = - \log (P)$$

where P is the probability that a Poisson spike train with the same mean firing rate would generate more spikes than emitted by the burst in the same time period. Bursts were identified as

a sequence of at least three inter-spike intervals (ISIs) with a Poisson surprise value >5 (P<0.00001). For each such burst, immediately-adjacent preceding and trailing spikes were added incrementally to maximize the Poisson surprise value. The onset and offset of a burst were set as the times of the first and last spikes of the burst. Figure 18B provides an example of this burst detection method in operation. The overall "burstiness" of a cell was quantified as the fraction of spikes that occurred during bursts relative to the total number of spikes in the cell's recorded spike train. The fraction of *time* each cell spent in bursts was also calculated. The two measures of burstiness (fraction of spikes and fraction of time in bursts) were found to correlate very closely with each other across cells (Spearman R=0.99, p<0.0001, not shown), indicating that the two measures were redundant. All subsequent analyses used fraction of spikes in bursts as the single measure of the overall amount of burst discharges ("burstiness") in a cell's spike train.

We examined the magnitude and timing of changes in a neuron's mean firing rate around the time of burst onset (i.e., burst morphology) by generating burst-triggered averages of a neuron's instantaneous firing rate (i.e., ISI^{-1}) for each cell in which >5 bursts were detected. To aid comparisons between cells, a cell's mean firing rate during the 200-msec prior to burst onset was subtracted from its peri-burst average.

A.2.5 Oscillatory Activity

Rhythmic modulations in neuronal firing rate (i.e., "oscillatory" firing) were quantified using a spike shuffling method (Rivlin-Etzion et al., 2006bb) designed to control for artifactual autocorrelations that arise from the neuronal refractory period. Neuronal spike times were represented as a delta function with a temporal resolution of 1-msec. The discrete Fourier

transform was applied to non-overlapping 2048-msec segments of the spike delta function smoothed with a Hanning window of the same length. This yielded spectral density estimates for frequencies between 0.25 and 500-Hz with a resolution of 0.5-Hz. Distortions of these estimates attributable to a neuron's refractory period were compensated for by dividing the actual spectrum by a normalizing spectrum, computed from the same data but after global-shuffling of the ISIs (Rivlin-Etzion et al., 2006bb). The normalizing spectrum was the mean of spectra computed from each of 1,000 random global shufflings of the same ISIs. Peaks in the normalized spectra between 0.25 and 200-Hz were tested for significance relative to the SD of the normalized spectra in the 300–500-Hz "control" range (Rivlin-Etzion et al., 2006bb). Peaks in the normalized spectrum were considered significant if they exceeded a threshold designed to yield an omnibus $p< 0.004$ (actual $p=1x10^{-5}$ after correcting for 400 comparisons). Cells with ≥ 1 significant peak(s) between 0.25–200-Hz were termed oscillatory firing (OF) cells. Autocorrelations were also computed to provide a qualitative assessment of a neuron's oscillatory firing.

A.2.6 Temporal relationship between burstiness and oscillatory activity

The temporal co-variation between bursts and oscillations was determined for each cell that exhibited at least one burst and one significant spectral peak above 2-Hz. We tested whether the prevalence of bursts and the strength of oscillatory firing co-varied in time across a cell's spike train. To obtain time-resolved estimates of the strength (power) of a cell's oscillatory firing, the cell's instantaneous firing frequency was first band-pass filtered using a filter custom-tuned to each cell's significant oscillatory frequency (Parks-McClellan optimal FIR in Matlab; pass-band and stop-band cutoffs ±1-Hz and ±2-Hz, respectively, relative to a significant spectral peak).

Figure 18B illustrates an example frequency of firing record before and after band-pass filtering. The band-pass range prevented analysis of oscillations <2-Hz. The resulting band-pass filtered signal was divided into 1-sec segments and root mean squared (RMS) power was computed separately for each segment. The burstiness of the cell's spike train (% of spikes in bursts) was also computed separately for each of the same 1-sec segments. [Parallel analyses were performed using other segment lengths (range 0.1-sec to 5-sec) and the results did not differ substantively.] These time-resolved measurements of RMS oscillatory power and burstiness were entered into a Spearman correlation from which the R value represented the degree to which a cell's oscillatory activity and burstiness co-varied in time across the recording. For cells with more than one significant spectral peak >2-Hz and at least one burst, time-resolved RMS power was computed and related to burstiness separately for each significant spectral peak. Note that at a fine temporal scale, bursts of neuronal activity always coincided with positive phases in the band-pass filtered signal such that the phase relation between bursts and oscillations was constant.

A.3 RESULTS

A.3.1 Cell database and general discharge characteristics

One-hundred and thirty-two GPi cells were studied in 14 PD patients. Recording durations of individual cells were 37.7±18-sec (mean +/- SD). Fifty-five cells were classified as oscillatory firing (OF) cells. The remaining 77 cells with no significant spectral peaks were classified as non-oscillatory firing (NOF) cells. Results from the oscillation analysis were summarized by

grouping OF cells by standard frequency bands [0.25-3 (delta), 3-8 (theta), 8-13 (alpha), 13-30 (beta), 30-60 (low gamma), >60-Hz (high gamma)] (Table 5).

Table 5: Firing properties of OF cells by frequency band

Frequency Band	Number cells (%)	Burstiness (%)	Firing Rate (spk/s)
All Frequencies	55 (100)	4.5 ±0.9	101 ±3.6
< 3 (delta)	24 (44)*	5.6 ±1.4	94.7 ±4.4
3-8 (theta)	25 (45)*	4.6 ±1.4	110.1 ±5.4
8-13 (alpha)	5 (9)	15.7 ±4.5†	86.8 ±9.2
13-30 (beta)	6 (11)	5.5 ±2.7	116.6 ±15.2
30-60 (low gamma)	3 (5)	1.6 ±1.1	87.3 ±8.9
> 60 (high gamma)	10 (18)	2.9 ±1.6	98.9 ±9.3

The number of cells (and percentage relative to all OF cells) with significant spectral peaks is indicated for each frequency band. Means ±SEM of the burstiness (% spikes in bursts) and average firing rates (Avg FR) are also provided for each frequency band. *, OF was more common at frequencies <8-Hz (chi-square test, p<0.01). †, burstiness was elevated in the five cells with alpha-range oscillatory firing when compared to all other OF cells combined (Mann-Whitney test, p<0.01). The number of cells listed in different frequency bands is greater than the total number of OF cells because 13 cells had more than one spectral peak.

A chi-squared analysis revealed that oscillatory firing occurred most often at frequencies below 8-Hz (χ^2=34.4, p<0.0001, 5 df). One-hundred and eight cells (81.8%) had at least 1 burst, while sixty-four cells (48.5%) had >5 bursts.

A.3.2 Bursts and oscillations as independent properties

Bursts and oscillations were found to be independent properties of spiking activity in the parkinsonian GPi. The fraction of cells with bursty firing (i.e., at least one burst) was very similar for OF neurons (44 of 55 cells, 80%) and NOF neurons (64 of 77 cells, 83%; χ^2=0.2, p>0.5; *Figure 19*A inset). Furthermore, most cells exhibited either bursts or oscillations, but not both.

Figure 18 illustrates the firing characteristics of a typical GPi cell that exhibited bursts without oscillations. Bursts (as detected by our algorithm) are identified by black horizontal lines below the rasterplot in Figure 18B and are sorted according to burst duration in the burst onset-aligned rasterplot in Figure 18E. Even though the activity of the exemplar cell in Figure 18 was quite bursty (28% of this cell's spikes occurred in bursts), its autocorrelation and power spectrum were notably flat (*Figure 18*D, *top and bottom*, respectively), providing no evidence for oscillatory modulations in firing.

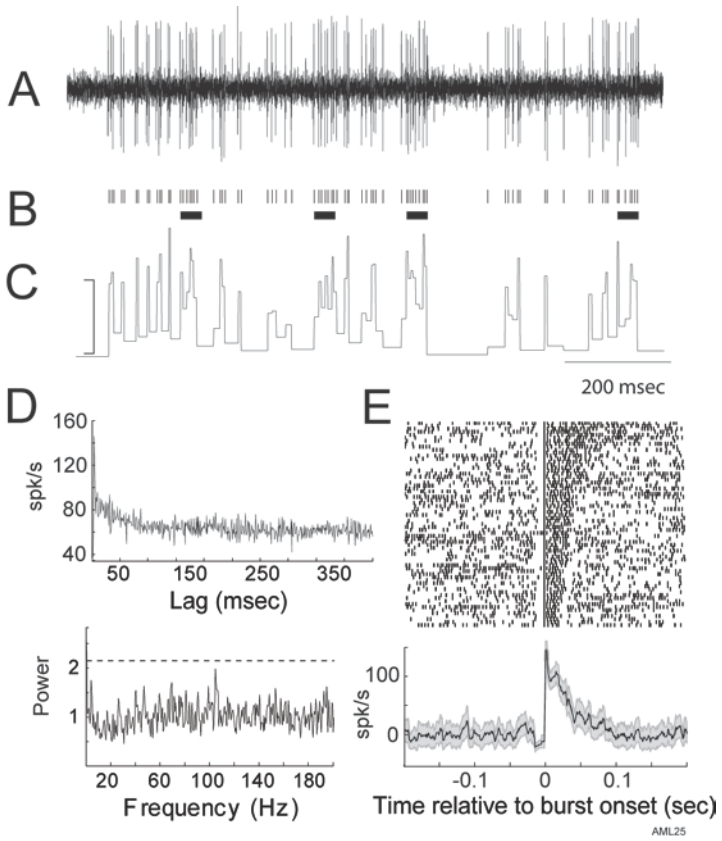

Figure 18: Example bursty cell

Exemplar data from an NOF bursting cell. A) A short segment of raw extracellular recording illustrating the well-isolated action potentials of a single neuron. B) After spike sorting, spike times were extracted and are represented here in a rasterplot. Bursts, as detected by our detection algorithm, are identified by horizontal black lines below the rasterplot. C) Instantaneous firing rate of this neuron during the same time segment (*calibration: 200-spk/s*). D) There was no evidence for oscillatory firing in either the autocorrelation (*top*) or normalized power spectrum (*bottom*) generated from all spikes from this neuron. The horizontal dotted line indicates the significance threshold. E) Peri-burst rasters from the same cell aligned on burst onset and sorted according to burst duration (*top*) with the mean peri-burst frequency of firing averaged across all bursts (*bottom*). Shaded *gray* region surrounding the peri-burst frequency of firing indicates the 95% confidence interval around the mean. Firing rates were normalized by the mean firing rate in the 200-msec preceding burst onset. Note the pre-burst "notch" in firing rate, the maximum intra-burst firing rate at burst onset, and the gradual decline in firing rate thereafter.

Other GPi cells (e.g., Figure 19) had no detectable bursts, but exhibited prominent oscillatory activity, as evidenced by periodicity in the auto-correlogram (*Figure 19*C) and a significant peak in the power spectrum (*Figure 19*D).

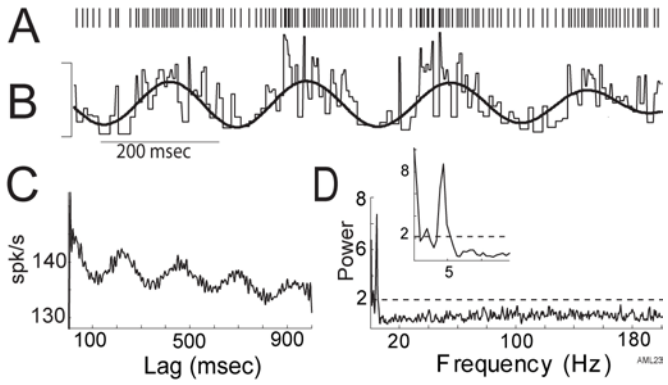

Figure 19: Example oscillatory cell

Oscillatory modulation in firing rate in the absence of burst firing. A) Rasterplot of a short segment of a spike train containing rhythmic modulations in firing rate but no burst firing. B) The raw instantaneous firing rate of this neuron (thin line) during the same time segment (calibration: 200-spk/s) overlaid by a band-pass filtered (4.4-Hz) version of the same instantaneous firing frequency (thick line). This spike train exhibited prominent oscillatory modulations in firing rate at 4.4-Hz, demonstrated by multiple peaks at regular intervals in the autocorrelation (C) and a significant peak centered on 4.4-Hz in the normalized power spectrum (D, exploded in inset).

Like the examples shown in Figures 18 and 19, most cells exhibited bursts without oscillations (64 cells), or oscillations without bursts (11 cells) (Figure 20A). These observations are inconsistent with the idea that oscillatory and bursty activity are closely-related phenomena in the parkinsonian GPi.

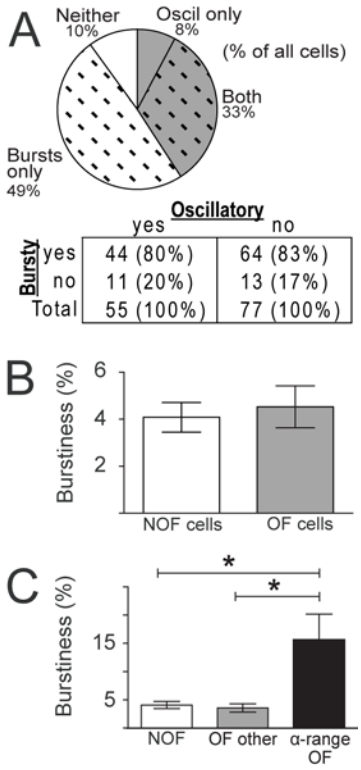

Figure 20: Bursts and oscillations as independent

A) Bursty firing (dashed hatching, Bursty "yes" in table inset) was found in equally large fractions of cells that had oscillatory firing (gray shading, Oscillatory "yes" in table inset) and cells that did not have oscillatory firing (Oscillatory "no" in table inset). B) The mean burstiness (% spikes in bursts) of OF cells was not significantly different from that of NOF cells (Mann-Whitney test, p=0.8). C) The mean burstiness of cells with spectral peaks in the *α*-range was significantly greater than that of NOF cells and all other OF cells (Kruskal-Wallis test, p<0.01). Columns represent mean +/- SEM. *denotes p<0.01 in Dunn's post tests.

The independence of bursting and oscillatory activity was substantiated further by comparing the mean burstiness of all OF cells with that of NOF cells. The mean fraction of spikes occurring in bursts for all OF cells (4.5% +/- 0.9; mean +/- SE) was very similar to that for NOF cells (4.1% +/- 0.6; Mann-Whitney test, *p*=0.8; Figure 20B). Therefore, burst firing occurred at statistically-indistinguishable levels in cells that displayed oscillatory activity and in cells that did not. Furthermore, across all OF cells, there was no general relationship between

the power of a cell's significant spectral peaks and the cell's burstiness (Spearman R=0.09, p=0.4; not shown).

A.3.3 Cells with alpha range oscillatory activity were burstier than other cells

Oscillatory activity within specific frequency bands is thought to be more "pathologic" than oscillatory activity in other frequency bands (Gatev et al., 2006; Levy et al., 2002ba; Rivlin-Etzion et al., 2006aa; Starr et al., 2005; Weinberger et al., 2006). Therefore, we explored the possibility that burstiness was more closely associated with oscillatory activity within specific frequencies. The mean burstiness values of OF neurons (% spikes in bursts) were compared across different frequency bands. For cells with oscillations in multiple frequency bands (n=13), the cell's burstiness value was included in each of those frequency bands. This analysis revealed substantially higher burstiness in cells that had ≥1 significant spectral peak in the α-range (15.7 +/- 5.4% spikes in bursts; mean +/- SEM, Figure 20C) compared with other OF cells (3.6% +/- 0.7) and NOF cells (4% +/- 0.6; Kruskal-Wallis test, p<0.01; Dunn's post-tests p<0.01 Figure 20C). Thus, OF cells with spectral peaks in the α-range were substantially burstier than all other cells. While only 4% of all cells (5 cells total) exhibited oscillatory activity in the α-range, these five cells were recorded from five different patients.

A.3.4 Unique burst morphology of alpha-range oscillatory cells

The unusually high burstiness of cells with α-range oscillatory activity prompted further analyses. Figure 21 depicts the firing characteristics of GPi cells with α-range oscillatory firing.

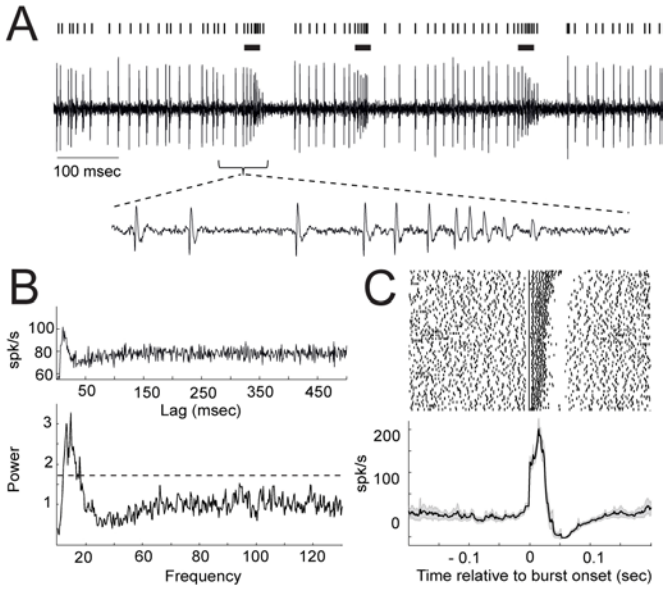

Figure 21: Example cell with alpha oscillatory activity

Cells with *α*-range OF exhibited elevated burst firing and a unique form of burstiness. A) A segment of raw data from an *α*-range OF cell with raster plot above indicating times of burst firing (*black horizontal lines*). A sub-panel below shows a time-expanded view of raw spike data from an exemplar burst to illustrate the accelerating firing rate during the burst accompanied by progressively decrementing action potentials. B) Marked modulations in the cell's autocorrelation (*top*) and significant spectral peaks in the *α*- and *β* frequency ranges of the power spectrum (*bottom*) are consistent with strong OF. C) Peri-burst rasters from the same cell aligned to the onset of the burst (*top*) and the mean peri-burst frequency of firing across all bursts (*bottom*). Otherwise, the figure follows the conventions outlined for Fig. 1. Note the absence of a pre-burst notch in firing rate, the accelerating firing rate during the burst, and abrupt cessation of firing with burst offset.

Comparison with Figure 18 draws attention to the unique features of bursting exhibited by *α*-range cells. Bursts in pallidal cells typically appear as an abrupt increase in firing rate preceded by a small reduction in firing (i.e., a longer than normal ISI; Figure 18E) (Wichmann and Soares, 2006). Additionally, firing rates within bursts usually peak at the time of burst onset and trail off smoothly to the baseline firing rate after that (Figure 18E *bottom*).

In contrast, the firing rates of α-range cells showed no evidence of a deceleration or pause preceding burst onset (Figure 21). Within bursts, these cells' firing rates accelerated progressively until the burst was terminated abruptly by a long-lasting cessation in firing (Figure 21A, C). This within-burst acceleration in firing rate was accompanied by a progressive diminution in action potential magnitude (Figure 21A *inset*), likely due to progressive failure of the cell's voltage-gated channels to reactivate fully.

The differences in burst morphology were substantiated by a comparison of population averages of peri-burst firing rates across the different cell categories (Figure 22).

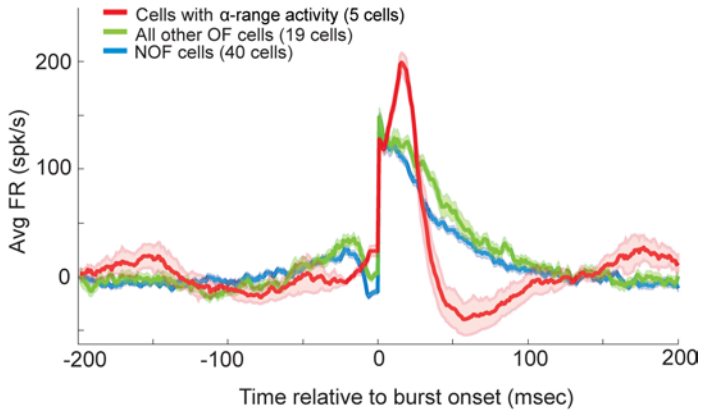

Figure 22: Peri-burst activity

Population averages of mean peri-burst frequency of firing for α-range OF cells, NOF cells, and all other OF cells with > 5 bursts. Firing rates were normalized individually by each cell's mean firing rate in the 200-msec preceding burst onset prior to averaging across cells. Shaded areas denote 95% confidence intervals for each population mean. Note the shorter burst duration and greater maximum intra-burst firing rate that occurred later in the bursts in α-range OF cells in comparison with all other OF and NOF cells.

Bursts in NOF cells and other (non-α-) OF cells showed a clear notch in firing rate immediately before burst onset, their peak intra-burst firing rate occurred at the time of burst onset, and they exhibited a gradual decline in firing rate thereafter. In contrast, bursts in α-range

OF cells showed no sign of a pre-burst notch, their firing rates accelerated during bursts, and they were followed by a marked post-burst decrease in firing rate. The bursts of α-range OF cells shared a strikingly similar morphology, as illustrated by the narrow 95% confidence intervals in Figure 22.

Quantitative analyses of burst metrics supported the differences in burst morphology described above. When compared with bursts in other cell populations, the bursts of α-range OF cells: (a) had a shorter duration, and a (b) greater maximum intra-burst firing rate (c) that peaked later in bursts (Kruskal-Wallis tests, all p values < 0.05; Table 6).

Table 6: Comparing peri-burst activity across cell populations

	Burst duration	**Max intraburst FR**	**Latency of Max FR**
NOF cells	46 +/- 2 ms*	163 +/- 7 spk/s*	8.7 +/- 1.6 ms *
Alpha range cells	32 +/- 3 ms	205 +/- 9 spk/s	17.6 +/- .7 ms
All other cells	53 +/- 3 ms*	162 +/- 5 spk/s*	8 +/- 2 ms *

Means ±SEM of burst duration, maximum intra-burst firing rate, and latency of maximum firing rate are provided for each category. *The cells with α-range activity had a shorter burst duration, and a longer maximum intra-burst firing rate that occurred later in the burst than other cells (Kruskal-Wallis test, $p<0.05$, Dunn's post test in comparison with α-range cells, $p<0.05$).

The distinctive burst properties of α-range OF cells could not be attributed to differences in the strength of oscillatory activity. The mean power of significant spectral peaks in the α-range was no different from that of significant peaks in any other frequency band (Dunn's post-test comparisons, $p>0.05$). It is also unlikely that this type of bursting activity was due to some form of injury discharge. The average recording duration for the five α-range cells was 51 +/-

7.5-sec. Visual inspection of the raw neuronal recording data confirmed that the similar unique bursting activity was present throughout the duration of each recording.

A.3.5 Burstiness, OF, and Firing Rate

Burstiness varied as an inverse function of a cell's mean firing rate within the general population of GPi neurons. There was a significant (p<0.0001; Spearman R=-0.48) negative correlation between a neuron's firing rate and its burstiness (Figure 23).

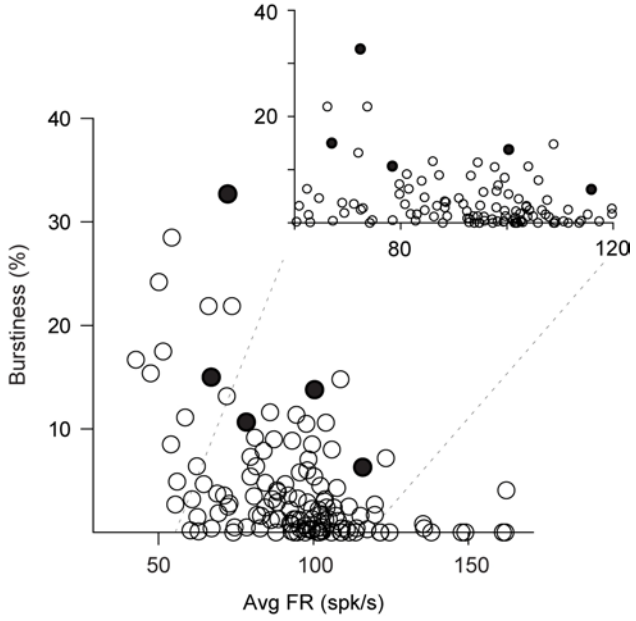

Figure 23: Relationship between average firing rate and burstiness

Burstiness was correlated inversely with mean firing rate (Spearman R=-0.48, p<0.0001). Each point in the graph represents one burst-firing cell. The values for α-range OF cells (*black circles)* fall within the general anti-correlated relationship identified for all bursty neurons.

The increased burstiness of α-range OF cells followed that general relationship. Notably, cells with α-range activity tended to be burstier (Figure 23, *black circles*) than other cells with similar firing rates (Figure 23, *white circles*).

Unlike burstiness, OF was associated with higher firing rates. Specifically, the mean firing rate of OF cells (101±3.6-spk/s; mean +/- SEM) was greater than that of NOF cells (89.7±2.1-spk/s; Mann-Whitney test, $p<0.01$). Interestingly, the mean firing rate of α-range OF cells (86.8±9.2 spk/s) was lower than that of other OF cells (102.4±3.8 spk/s) and even of NOF cells (89.7±2.1 spk/s) although those differences were not significant due to the small number of α-range OF cells (Dunn's post test $p>0.05$).

A.3.6 Weak temporal co-variation between bursts and oscillations in individual cells

To test the independence of bursts and oscillations further, we quantified relationships between the strength of oscillatory activity and burstiness across time in individual neurons. The degree to which burstiness and oscillatory power co-varied across time was quantified by correlation analysis for each cell with bursts and oscillations (>2–Hz). A minority of cells (34/132) displayed both burst firing (≥1 bursts) and oscillatory activity >2-Hz. Second-by-second burstiness values ranged between 0-50% in 85% of these cells, suggesting that burstiness varied adequately across time to allow detection of temporal correlations if they were present. Time-resolved oscillatory power and burstiness were not correlated in 20 of those 34 cells (Spearman correlations, $p>0.05$), as demonstrated by an example cell in Figure 24A (Spearman $R^2=7\%$, $p>0.05$).

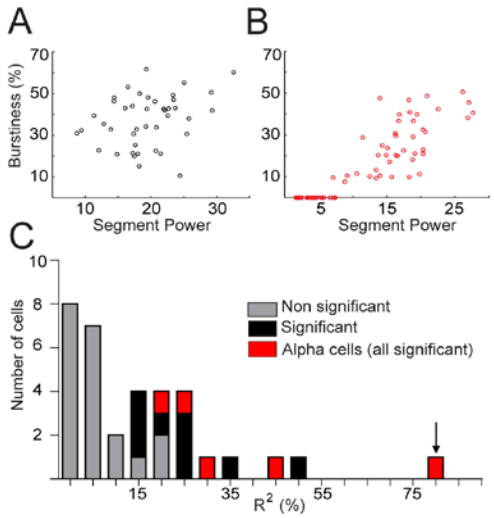

Figure 24: Relationship between oscillatory and bursty activity

A) Correlation analysis revealed a lack of any relationship between RMS power and burstiness in most cells, such as the example cell shown here (Spearman correlation, R^2=7%, *p*>0.05). B) Some cells exhibited a positive correlation between RMS power and burstiness. Burstiness (% spikes in bursts) is plotted versus mean RMS power (8.3-Hz in this case), depicting the relationship for the highest R^2 value detected from any correlation (*red, B*; *arrow, C;* Spearman correlation, R^2=78%, *p*<0.01). Note that this cell happens to be an *α*-range OF cell. C) Identical correlation analyses were performed for all cells with bursts and oscillations >2-Hz. This figure depicts relationships between oscillatory activity and burstiness across different frequency ranges. Each point represents an R^2 value for each significant oscillatory peak detected. Therefore, if a cell exhibited multiple oscillatory peaks, multiple points represent this cell. Note that the oscillations in all *α*-range OF cells (*red stars*) but one (*black star)* had significant and positive correlations and some of the highest R^2-values.

Among the 14 cells that did show significant correlations with one or more of their oscillatory peaks ($p<0.05$), the correlations were typically positive, but weak, with variations in oscillatory power accounting for $\leq$40% of the variance in burstiness in most cells (Spearman correlations, Figure 24C). Interestingly, *α*-range OF cells exhibited some of the strongest

correlations. Figure 24B illustrates the strongest temporal relationship identified between burstiness and oscillatory activity (8.3-Hz in this case; *arrow*, Figure 24C*)*.

A.3.7 Clinical Relationships

All patients were tremulous with one exception. Bradykinesia and rigidity were also present in every patient. Total UPDRS scores ranged from 31-66 (43.3 +/- 3.1; mean +/- SEM). Responsiveness to DA therapy ranged from 30% to 86% (54 +/- 6%). We found no significant relationship between the power of oscillatory firing, burstiness, or mean firing rate and either UPDRS scores or responsiveness to DRT (% change in UPDRS; Spearman correlations, all p values>0.5). UPDRS scores and responsiveness to DRT were similar in patients with α-range OF cells and those in whom none were found (Mann-Whitney tests, all p values>0.5).

A.4 DISCUSSION

A.4.1 Bursts and Oscillations as Independent Properties

Neuronal activity in the parkinsonian BG is characterized by an increased prevalence of oscillatory firing and bursting activity, leading many to assume that the two are closely linked phenomena. To the best of our knowledge, that assumption has not been tested empirically. Here we found little support for the idea. Burst firing (≥1 burst) was found in equally large fractions of cells regardless of whether they were OF or NOF cells. Furthermore, cells with oscillatory activity were characterized by a level of burstiness (mean % of spikes in bursts) that

was statistically-indistinguishable from that of NOF cells. Even within the subset of cells that exhibited both bursts and oscillations, second-to-second changes in burstiness and oscillatory power were seldom strong predictors of each other. Together, these results are consistent with the idea that bursts and oscillations are, for the most part, independent pathophysiologic features of the parkinsonian GPi. These observations cast doubt on earlier hypotheses that oscillatory activity in the BG is generated by rebound bursts in the subthalamic nucleus (STN) (Plenz and Kital, 1999) that trigger additional bursts in other BG regions, creating self-sustaining oscillatory activity throughout the BG (Galvan and Wichmann, 2008). Instead, we found that the activity of many cells was characterized exclusively by bursts *or* oscillations, and that NOF cells were just as bursty as OF cells.

The presence in some neurons of correlated modulations in burstiness and oscillatory power (Figure 24) indicates that the two phenomena may wax and wane together in a subset of cells. Most temporal correlations were relatively weak, however, and were significant only in a minority of the neurons (14/34). In general, burstiness and oscillatory power varied independently across time within individual neurons, even on a 1-sec time scale.

These findings, however, are subject to potential confounds. The activity of some cells may have been characterized by brief epochs of oscillatory activity without meeting the standards set here to identify significant oscillatory activity. In such situations, temporal relationships between bursts and oscillatory activity would not have been detected, causing any relationship between bursty and oscillatory activity to be underestimated. Oscillatory firing and bursts might be more closely linked in a subset of GPi neurons that were not encountered here due to under-sampling of the GPi. In addition, the specific algorithm and statistical criteria used for burst detection could have biased our classification of bursts, thereby altering the

identification of relationships between bursts and oscillations. This work, however, employed detection methods for bursts and oscillatory activity that are both widely-accepted and frequently used to characterize pathophysiologic activity in the parkinsonian state (see above). Thus, even with these potential confounds, the current findings are likely to be relevant to our current understanding of bursts and oscillatory activity in the basal ganglia, and how they contribute to the pathophysiology of PD. Furthermore, these findings are in agreement with our previous work. Intrastriatal DA receptor blockade increased oscillatory activity of cortical cells without causing a significant increase in burstiness (see above).

A.4.2 Oscillatory firing, burstiness, and firing rate

Oscillations and bursts were not only found to be relatively independent of each other, but were also differently related to other properties of neural activity, such as firing rate. OF cells exhibited higher mean firing rates than NOF cells, consistent with the idea that BG oscillations are adopted from cortical oscillations when dopamine deficiency leads to a higher background firing rate (Levy et al., 2002aa). That observation is also consistent with the premise that OF superimposed on a high frequency baseline firing rate is a feature of the parkinsonian BG (Starr et al., 2005).

The higher firing rates observed in OF cells may be partly due to inherent biases of our detection methods, however. Oscillatory firing is more likely to be detected in cells with higher FR due to increased signal. Moreover, the statistical power for detecting OF is inherently weaker in cells with lower firing rates, especially given the short duration of many of our

recordings. While these potential confounds are important to consider, the high firing rates of all GPi cells makes it unlikely that differences in firing rate dramatically biased the detection of OF.

In contrast to OF, mean firing rates were *negatively* correlated with burstiness. That result was somewhat surprising, as bursting activity is known to coexist with oscillatory activity and increased baseline firing in the parkinsonian GPi (Filion and Tremblay, 1991; Raz et al., 2000; Starr et al., 2005; Wichmann and Soares, 2006). Importantly, however, this finding is consistent with the idea that oscillations and bursts are unrelated phenomena in the parkinsonian GPi.

A.4.3 Cells with alpha-range OF differ from other cells in burstiness and burst morphology

While oscillations and bursts emerged as generally independent properties, GPi cells with α-range oscillatory activity were found to be much burstier than all other cells. The morphology of bursts in these cells differed markedly from the typical burst firing described for the parkinsonian GPi (Wichmann and Soares, 2006). One previous study mentioned a similar form of burst firing in the GPi of PD patients ("GPi tonic bursters", (Taha et al., 1997). To our knowledge, the present study is the first to characterize this type of burst activity in detail, observe that it is strongly associated with α-range OF, and find that it is not attributable to differences in spectral power or firing rate. Cells with α-range OF also exhibited burst firing with decrementing action potentials, a well-established characteristic of a sub-population of neurons in the external segment of the globus pallidus [GPe, “LFD-B” neurons, (DeLong, 1971)]. Although only 5/132 cells displayed α-range OF, the increased burstiness and burst morphology of these five cells was remarkably similar to each other and substantially different from all other cells. It is unlikely

that this form of burst firing was some type of injury discharge because its presence was stable across the duration of recordings. Alpha-range OF was found in five different patients and thus was not an anomaly specific to on individual.

Future studies are needed to explore the role of α-range oscillatory cells in the pathophysiology of PD. Modeling studies (Rubin and Terman, 2004; Terman et al., 2002) and *in vivo* experiments (Kojima and Doupe, 2009; Person and Perkel, 2005) suggest that BG-recipient thalamic neurons are particularly susceptible to driving by a burst-pause pattern of BG input such as that exhibited by α-range OF cells. Indeed, several studies indicate that the suppression of α-range oscillations in the BG is associated with therapeutic benefit (Heimer et al., 2006; McCairn and Turner, 2009).

A.5 CONCLUSIONS AND FUTURE DIRECTIONS

Parkinsonism has long been associated with pathologically-increased levels of oscillatory and bursty activity in the GPi. Therefore, those two features of neuronal activity have often been lumped together as linked contributors to the pathophysiology of PD. By examining the prevalence of bursts and oscillations on a single cell level, however, we found that oscillatory and bursty activity are generally independent properties of spiking activity in the parkinsonian GPi. Therefore, bursts and oscillations may well play functionally-distinct roles in the genesis of PD motor signs, and may even represent separate therapeutic targets or indicators of responsiveness to therapy. In contrast to the general independence of bursts and oscillatory firing, the activity of cells with α-range oscillations was uniquely bursty with a distinct burst morphology. The present work emphasizes the importance of considering bursts and oscillations

independently in future studies of the neurophysiologic correlates of PD. Further studies are needed to relate bursts and oscillations independently to the development and resolution of specific parkinsonian signs.

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