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Characteristics of Tremorogenesis and Tremorolytic Agents in a Pharmacological Rodent Model of Parkinsonism: Evidence from Behavioral, Neurochemical, and Electrophysiological Studies

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Characteristics of Tremorogenesis and Tremorolytic Agents in a Pharmacological Rodent Model of Parkinsonism: Evidence from Behavioral, Neurochemical, and Electrophysiological Studies

Samantha J. Podurriel, PhD

University of Connecticut, 2015

The central aim of the present research was to characterize aspects of the instigation and treatment of Parkinsonian resting tremor using a pharmacological rodent model, the tremulous jaw movement (TJM) model. These studies employed the TJM model to investigate the neurochemical and electrophysiological changes that are associated with tremorogenesis, and to test novel therapeutic agents for the treatment of resting tremor. Experiment 1 established a mouse model of Parkinsonian resting tremor using the anticholinesterase galantamine. In experiment 2, the novel antiparkinsonian agent safinamide attenuated TJMs induced by the anticholinesterase galantamine, the muscarinic agonist pilocarpine, and the dopamine D2 antagonist pimozide. In experiment 3, the MAO-B inhibitor deprenyl attenuated TJMs induced by the VMAT-2 inhibitor tetrabenazine (TBZ). TBZ administration decreased extracellular DA levels in the ventrolateral striatum (VLS), and co-administration of deprenyl blunted this effect. The aim of experiment 4 was to characterize the effect of the SSRI fluoxetine (FLX) on the motor dysfunctions induced by TBZ and investigate the neural mechanisms involved. Co-administration of FLX increased TBZ-induced TJMs and decreased locomotor activity compared to TBZ alone. Co-administration of the 5-HT_{2A/2C} antagonist mianserin attenuated the increase in TJMs induced by co-administration of TBZ with FLX. Co-administration of TBZ and FLX decreased DA tissue levels in the rat VLS compared to TBZ alone, and co-administration of mianserin with TBZ and FLX attenuated this effect, increasing DA tissue levels compared to the TBZ/FLX condition. The fifth set of studies examined if tremor-related local field potential (LFP) activity could be recorded from motor cortex or subthalamic nucleus (STN) during TJMs

induced by the muscarinic agonist pilocarpine. Pilocarpine induced a robust TJM response that was marked by rhythmic electromyographic activity in the temporalis muscle. TJM epochs were characterized by increased LFP power in the tremor frequency range in both neocortex and STN. Tremor activity was not associated with increased power in the beta frequency band. These studies collectively extended and validated the TJM model, contributing to the ultimate goal of this line of research, which is to characterize the conditions associated with tremorogenesis in order to develop specifically-targeted therapeutic strategies.

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of Parkinsonism: Evidence from Behavioral, Neurochemical, and Electrophysiological Studies

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B.A., University of Connecticut, 2010

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Doctor of Philosophy Dissertation

Characteristics of Tremorogenesis and Tremorolytic Agents in a Pharmacological Rodent Model
of Parkinsonism: Evidence from Behavioral, Neurochemical, and Electrophysiological Studies

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Chapter 1: Parkinson's Disease: Pathology, Symptoms, and Treatments

Parkinson's Disease and Parkinsonism

There are currently 1 to 2 million cases of Parkinson's Disease (PD) in the United States and several million worldwide, making it the second most common neurodegenerative disorder (Nussbaum and Ellis, 2003; Ostrem and Galifianakis, 2010). Age is considered the strongest risk factor for PD, as it affects 1-2% of the population over the age of 65, and more than 3% of those over the age of 85. Given the current aging of the population, both the prevalence of PD and its national economic burden are expected to increase substantially as the population continues to age (Ostrem and Galifianakis, 2010; Shulman et al., 2011; Kowal et al., 2013). Conservative projections suggest that the number of individuals diagnosed with PD will likely double between 2010 and 2040 (Kowal et al., 2013). PD is typically diagnosed between age 70 and 80 and progresses chronically and slowly until death, on average 15 years after initial diagnosis (Shulman et al., 2011). Upon postmortem examination of brains of PD patients, researchers have identified the presence of Lewy bodies and Lewy neurites (filamentous, α -synuclein-rich protein aggregates found in the cytoplasm and processes, respectively, of neurons; Nussbaum and Ellis, 2003). However, the neuropathological hallmark of PD is the death of dopamine (DA) producing cell bodies in the substantia nigra *pars compacta*, which causes the degeneration of the nigrostriatal pathway and depletion of DA in striatal areas (Hornykiwicz, 1973). According to models of basal ganglia function, this degeneration of nigrostriatal neurons input leads to a net increase in inhibitory output from the globus pallidus interna (i.e., medial globus pallidus) and the substantia nigra pars reticulata, ultimately affecting the function of the motor cortex and brainstem motor areas (Hornykiwicz, 1973; Shulman et al., 2011).

Idiopathic PD is a member of a broader family of movement disorders known as Parkinsonism. While idiopathic PD is by far the most common form of Parkinsonism, drug administration is the second most common (Alvarez et al., 2007; Shulman et al., 2011). Drug-induced Parkinsonism (DIP) can result from administration of pharmacological agents that interfere with DA transmission such as DA antagonists (e.g. haloperidol, pimozide) and DA depleting agents (e.g. reserpine, tetrabenazine) (Marsden et al., 1975; McEvoy, 1983; Arbaizar et al., 2008). Thus, DIP has been associated with the administration of a variety of typical and atypical antipsychotics, antiemetics, antihypertensives, gastrointestinal prokinetics, and antivertigo agents (Mena and de Yébenes, 2006; Alvarez et al., 2007; Shin and Chung, 2012). Additionally, cholinomimetic administration can induce or exacerbate Parkinsonian motor symptoms. Anticholinesterases are the primary treatment for Alzheimer's disease, and several clinical studies indicate that patients have developed parkinsonian motor side effects (Iwasaki et al. 1988; Ott and Lannon, 1992; Kao et al., 1993; Keltner, 1994; Aarsland et al. 2003).

Parkinsonian Resting Tremor and the Tremulous Jaw Movement Model

Parkinsonism is characterized by several cardinal motor symptoms: resting tremor (3-7 Hz), bradykinesia (slowed movement), akinesia (lack of initiation of spontaneous movement), and rigidity (increased muscular tone, passive resistance to movement) (Marsden et al., 1975; Findley, 1988; Bergman et al., 2002, Ostrem and Galifianakis, 2010; Shulman et al., 2011). Parkinsonian resting tremor, defined as a “rhythmic, oscillatory, involuntary movement,” (Ostrem and Galifianakis, 2010) is the most common hyperkinetic movement associated with parkinsonism, and occurs in a frequency range of 3-7.5 Hz, which is distinct from dyskinesias (1-2 Hz), essential tremor (8 Hz), and postural tremors (8-12 Hz) (Findley et al., 1981, Marsden,

1984; Deuschl et al., 1996, 2000). Parkinsonian resting tremor most frequently presents unilaterally in the distal upper extremities as a “pill rolling” movement, but typically spreads bilaterally, affecting the upper and lower limbs, facial muscles, and the jaw, a condition known as “rabbit syndrome” (Weiss et al. 1980; Salamone et al., 1998; Deuschl et al., 2000; Ostrem and Galifianakis, 2010).

Rodent models have been essential to the scientific study of PD, and are frequently used to investigate both the underlying pathology and novel treatment strategies (Cenci et al., 2002; Betarbet et al., 2002). A number of rodent tests exist to assess the motor functions associated with akinesia and bradykinesia (e.g. catalepsy and locomotor activity assessment), yet resting tremor is the most common and easily recognizable symptom of PD and, thus, has been increasingly studied in recent years (Jankovic, 2008; Ostrem and Galifianakis, 2010). Currently, the tremulous jaw movement model is the most widely used rodent model for studying parkinsonian tremor. Tremulous jaw movements (TJMs) are defined as “vertical deflections of the lower jaw that resemble chewing but are not directed at any particular stimulus” (Salamone et al., 1998, 2005). In rats, the ventrolateral neostriatum (VLS), the striatal region responsible for orofacial movements and forepaw motor control, is the striatal region associated with the production TJMs (Salamone et al., 1990; Jicha and Salamone, 1991; Salamone et al., 1993). Local infusion of the neurotoxin 6-hydroxydopamine (6-OHDA) into the VLS has been shown to induce TJMs, while infusion into other striatal areas (e.g. anteroventromedial striatum, dorsolateral striatum, nucleus accumbens) does not (Finn et al., 1998; Jicha and Salamone, 1991).

TJMs in rats can also be induced by many of the same pharmacological agents that are responsible for DIP in humans. Interference with DA transmission, through the administration of

either DA antagonists (e.g. pimozide, haloperidol) or DA-depleting agents (e.g. reserpine, tetrabenazine), has been extensively shown to result in the production of TJMs in rats (Baskin and Salamone, 1993; Steinpreis et al., 1993; Salamone and Baskin, 1996; Salamone et al., 1998; Correa et al., 2004; Ishiwari et al., 2005; Salamone et al., 2008; Betz et al., 2009; Podurgiel et al., 2013a). Tetrabenazine (TBZ) is a reversible inhibitor of vesicular monoamine transporter 2 (VMAT-2) that is prescribed for treatment of chorea associated with Huntington's disease (HD). VMAT-2 is present in the brain and is responsible for transporting catecholamines from the cytoplasm into synaptic vesicles. TBZ has the highest binding density in DA-rich areas of the brain including the caudate, putamen, and nucleus accumbens (Pettibone, 1984a, 1984b; Thibaut, 1995) and thus inhibits the storage of DA, resulting in profound DA depletion. Accordingly, Parkinsonism is one of the most common adverse effects observed in HD patients taking TBZ for the treatment of chorea (Kenney et al., 2007; Frank, 2009). TBZ administration in rats has recently been established as a pharmacological model of Parkinsonism, as it induces TJMs and catalepsy and suppresses locomotor activity (Podurgiel et al., 2013a). Alterations in acetylcholine (ACh) transmission via administration of muscarinic agonists (e.g. pilocarpine) and anticholinesterases (e.g. tacrine, galantamine) have also been implicated in the production of TJMs (Salamone et al. 1986; Baskin et al., 1994; Mayorga et al., 1997; Salamone et al., 1998; Collins et al., 2010a; Collins et al., 2011). Studies employing freeze frame video analysis show the local frequency of TJMs is 3-7.5 Hz, which corresponds to the frequency range characteristic of Parkinsonian resting tremor (Salamone et al. 1996, 1998; Finn et al., 1997; Mayora et al., 1997; Cousins et al., 1998; Ishiwari et al., 2005; Collins et al., 2011). Furthermore, electromyography (EMG) recordings from the lateral temporalis muscle (the jaw-closing muscle that has been implicated in TJM activity) in rats show consistent, rhythmic bursts of EMG

activity in the 4-5 Hz frequency range during burst TJMs, again consistent with the frequency range of Parkinsonian resting tremor (Cousins et al., 1998; Collins et al., 2011). TJMs in rats can be attenuated using antiparkinsonian agents including L-DOPA (Cousins and Salamone, 1996; Cousins et al. 1997) DA agonists (Baskin and Salamone, 1993; Cousins et al., 1997; Salamone et al., 2005) amantadine (Cousins et al., 1997), muscarinic antagonists (Steinpreis et al., 1993; Cousins et al., 1997; Mayorga et al., 1997; Betz et al., 2009), and adenosine A_{2A} antagonists (Correa et al. 2004; Salamone et al., 2008; Betz et al., 2009; Collins et al., 2010a, 2011, 2012; Salamone et al., 2013; Santerre et al., 2012; Podurgiel et al., 2013a). Furthermore, TJMs in rats can also be attenuated by deep brain stimulation of the subthalamic nucleus (STN) (Collins-Praino et al., 2013).

More recently, researchers have extended the TJM model to mice, thereby providing a platform for examining the effects of various genetic manipulations on parkinsonian tremor. Like rats, TJMs are induced in mice after administration of the DA-depleting agent tetrabenazine (Podurgiel et al., 2013), and the muscarinic agonist pilocarpine (Salamone et al., 2013); the adenosine A_{2A} antagonist MSX-3 is capable of attenuating TBZ and pilocarpine-induced TJMs (Salamone et al., 2013; Podurgiel et al., 2013). Pilocarpine-induced TJMs in mice also occur primarily in bursts with a peak frequency of 3-7.5 Hz (Salamone et al., 2013). Furthermore, adenosine A_{2A} receptor knockout (KO) mice show a reduction in TJMs after administration of TBZ (Podurgiel et al., 2013) or pilocarpine (Salamone et al., 2013) compared to wild type (wt) controls. Thus, extending the TJM model to mice has given researchers means to investigate a new dimension of Parkinsonism and tremorogenesis.

Pharmacological Treatment Regimens for the Motor Symptoms of PD

3,4-dihydroxy-L-phenylalanine (levodopa or L-DOPA) is a naturally occurring amino acid precursor to DA that was first given to PD patients in 1961 (Tolosa et al., 1998). After oral ingestion, L-DOPA is actively transported from the small intestine into systemic circulation, and once it crosses the blood brain barrier (BBB) it is rapidly converted to DA via the enzyme aromatic L-amino acid decarboxylase (AAAD) (LeWitt, 2008). In order to inhibit the conversion of L-DOPA to DA outside the central nervous system, L-DOPA is often coadministered with a peripherally acting AAAD inhibitor (either carbidopa or benserazide) (LeWitt, 2008).

Coadministration of levodopa and an AAAD inhibitor remains the most effective medication available for the treatment of the motor symptoms of PD (Pedrosa and Timmermann, 2013).

After oral ingestion, patients typically experience the therapeutic effect within 15 to 30 minutes marked by improvements in speech, dexterity, and gait (LeWitt, 2008). Patients in the early stages of PD experience consistent, sustained therapeutic responses to levodopa that lasts until the next dose (Ostrem and Galifianakis, 2010). However, within 5 years up to 50% of patients taking levodopa develop motor fluctuations (i.e., rapid fluctuations between the “on” state where patients show good response to the medicine, and the “off” state where they show little to no response to the medication) dyskinesias, or both (Nutt, 2001; Holloway et al., 2004; Stacy, 2009).

DA agonists (e.g. pramipexole, ropinerole) have been used regularly to treat the motor symptoms of PD since the discovery of the benefits of bromocriptine in 1974 (Tolosa et al., 1998). DA agonists are often prescribed to patients in the early stages of PD as a monotherapy or in conjunction with levodopa (Pedrosa and Timmermann, 2013). When DA agonists are used

as an adjunctive therapy, PD patients experience a 20% to 40% reduction in off states, equal to an average of 2 hours per day (Bonuccelli, 2003).

Monoamine oxidase type B (MAO-B) is one of the key enzymes responsible for DA metabolism in the brain (Youdim and Bakhle, 2006). Deprenyl (Selegiline; ((R)-(-)-N- α -Dimethyl-N-2-propynylbenzeneethanamine hydrochloride; ((R)-(-)-Deprenyl) is a potent, irreversible MAO-B selective inhibitor that been available for over 30 years for the treatment of motor symptoms in early and late stage PD (Schapira et al., 2011; Fabbrini et al., 2012). Deprenyl is well tolerated in PD patients, with side effects/adverse reactions (e.g. sleeplessness, nausea, vomiting, dizziness, dry mouth, orthostatic hypotension and dyskinesias) occurring in 2-5% of patients, levels comparable to placebo (Riederer and Laux, 2011). When taken as a monotherapy in early PD, deprenyl delays the need for the introduction of L-DOPA (Fabbrini et al., 2012). As an adjunct treatment to L-DOPA, deprenyl prolongs the effectiveness of L-DOPA while reducing motor fluctuations (Riederer and Laux, 2011). Furthermore, deprenyl as an adjunct to L-DOPA enhances cognition, affect, and quality of life compared to L-DOPA or DA agonists alone (Krishna et al., 2014). Safinamide ((S)-(+)-2-[4-(3-fluorobenzyloxybenzylamino)pro-panamide]methanesulfonate (1:1 salt) is a potent, highly selective and reversible inhibitor of MAO-B that also reduces DA uptake, blocks voltage-dependent sodium channels, modulates N-type calcium channels, and reduces glutamate release (Marzo et al. 2004; Fariello 2007; Onofrj et al. 2008; Schapira 2010; Stocchi 2012). By doing so, animal studies have revealed that safinamide exerts neuroprotectant, anticonvulsant, and antiparkinsonian properties (Salvati et al. 1999; Fariello et al. 2000). Safinamide has an excellent therapeutic and safety margin and is currently in Phase III clinical trial development as an add-on therapy to levodopa or DA agonists for PD patients (Marzo et al. 2004; Onofrj et al. 2008; Schapira 2010; Stocchi 2012).

Adenosine A_{2A} receptors are densely concentrated in the striatum where they are colocalized with DA D2 receptors on enkephalin-positive medium spiny neurons (Ferre et al., 1997, 2001). Research has shown that these receptors converge on the same cAMP-associated signal transduction mechanism and exert opposing effects (Ferre et al., 2008). DA D2 receptors are linked to the g-protein G_i and inhibit adenylate cyclase, while A_{2A} receptors are linked to the g-proteins G_s/G_{olf} and activate adenylate cyclase (Ferre et al., 1997; 2001, 2008; Iversen et al., 2009). This interaction has led researchers to investigate the use of adenosine A_{2A} antagonists as a non-dopaminergic treatment for the motor symptoms of PD. Studies in rodents have shown that A_{2A} antagonists are capable of attenuating tremulous jaw movements induced by various DA-depleting agents, DA antagonists, and cholinomimetics (Correa et al. 2004; Salamone et al., 2008; Betz et al., 2009; Collins et al., 2010a, 2011, 2012; Salamone et al., 2013; Santerre et al., 2012; Podurgiel et al., 2013a). Recently, istradefylline (KW6002) became the first A_{2A} antagonist to undergo clinical trials for treatment of PD. In double-blind, placebo-controlled studies in levodopa-treated PD subjects with motor fluctuations, istradefylline-treated patients experienced a significant reduction in the amount of time spent in the off state compared to the placebo treated group (Hauser et al., 2003; Mizuno et al., 2010). Similar results have been obtained with the A_{2A} antagonist preladenant (Hauser et al., 2011). Istradefylline is now used clinically in Japan for the treatment of Parkinson's disease.

SSRIs for the Treatment of Depression in PD Patients

While Parkinsonism is primarily characterized by the generation of the cardinal motor symptoms, patients with PD also suffer from a variety of significant non-motor symptoms, including autonomic dysfunction, sensory abnormalities, gastrointestinal issues, sleep disorders, and neuropsychiatric disturbances (Chaudhuri et al., 2006; Ostrem and Galifianakis, 2010; Barone, 2011). Neuropsychiatric symptoms are common even in the earliest stages of the disease, and can considerably affect the daily functioning and overall quality of life of PD patients (Chaudhuri et al., 2006; Aarsland et al, 2009; Barone, 2011). Depression, in particular, has been identified as the most significant predictor of health-related quality of life in PD (Schrag et al., 2000; Chaudhuri et al., 2006; Chen and Marsh, 2013), and systematic review and analysis suggest that 35-40% of patients with PD also experience clinically significant symptoms of depression (Slaughter et al, 2001; Aarsland et al, 2009).

Selective serotonin reuptake inhibitors (SSRIs) are prescribed more often than any other class of antidepressants for PD patients (Vaswani et al, 2003; Veazey et al, 2005; Aarsland et al, 2009; Chen and Marsh, 2013; Schreiber and Thompson, 2013). Yet controlled clinical trials, meta-analyses, and systematic review collectively suggest that SSRIs are no more effective than placebo in treating depression in the context of PD (Skapinakis et al, 2010; Aarsland et al, 2009). Furthermore, SSRI administration has been associated with a number of motor side-effects, and may be implicated in increased motor disability in PD patients (Leo, 1996; Richard et al, 1997; Gerber and Lynd, 1998; Govoni et al, 2001; Vaswani et al, 2003; Veazey et al, 2005; Aarsland et al, 2009). There are presently more than 100 published reports of “extrapyramidal” symptoms (e.g. dystonia, akathisia, dyskinesia, and Parkinsonism, including tremor) associated with SSRI

treatment; fluoxetine (Prozac; FLX) has been implicated in the majority of these reports (Madhusoodanan et al, 2010).

FLX primarily functions as an inhibitor of the serotonin (5-HT) transporter, preventing uptake of 5-HT and ultimately resulting in increased activation of a variety of 5-HT receptors, including the 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors found throughout the striatum (Nutt et al, 1999; Alex and Pehek, 2007; More et al, 2014). Interactions between 5-HT and DA neurotransmission are strongly implicated in the generation of motor dysfunctions associated with FLX treatment (Morelli et al, 2011), as DA release and metabolite production are inhibited by increased synaptic levels of 5-HT (Govoni et al, 2001; Morelli et al, 2011). Activation of 5-HT_{2C} receptors has been linked to decreased DA synthesis, neural activity and release in the nigrostriatal and mesolimbic DA pathways (Alex and Pehek, 2007; More et al, 2014). In rodent models, FLX administration has been shown to potentiate haloperidol-induced catalepsy and bradykinesia in a dose-dependent manner (Tatara et al, 2012; More et al, 2014).

Cortical and Subthalamic Oscillatory Activity in PD Patients

Exaggerated neuronal synchrony has been observed within the basal ganglia of patients with PD, indicated by increases in oscillatory activity in the discharge of single neurons, increased amplitude of local field potentials (LFPs), and increased coherence of LFP signals across different basal ganglia structures in patients undergoing surgical procedures (Brown et al., 2001; Brown and Williams, 2005; Hammond et al., 2007; Gale et al., 2008). There is oscillatory activity at a wide range of frequencies within basal ganglia circuits, ranging from slow (< 2 Hz) oscillations to faster gamma frequency (30-80 Hz) oscillations (Pare et al., 1990; Brown, 2003).

The best-characterized oscillation frequency band in the basal ganglia of PD patients is the beta band (~15-30 Hz), as indicated by the LFP in basal ganglia structures, as well as cortical electroencephalogram (EEG) and magnetoencephalography (MEG) (Brown et al., 2001; Priori et al., 2004; Hammond et al., 2007; Hirschmann et al., 2011; George et al., 2013; Oswal et al., 2013). Increased oscillatory activity in the beta band has been observed in the subthalamic nucleus (STN) of PD patients, and it has been suggested that excessive synchrony in this frequency range contributes to deficits in motor control (Levy et al., 2002; Brown and Williams, 2005; Kuhn et al., 2006). Increases in STN beta power can be observed in the LFP of PD patients off L-DOPA, which diminishes once L-DOPA treatment is reinstated (Brown et al., 2001; Levy et al., 2002; Priori et al., 2004; Brown and Williams, 2005). Furthermore, improvement in akinesia-rigidity as indicated by the Unified Parkinson's Disease Rating Scale (UPDRS) in PD patients is highly correlated with reduction in STN beta band power (Kuhn et al., 2006). Thus, reduction in beta band oscillatory activity in the STN is thought to correlate with motor symptom improvement, particularly akinesia and rigidity, in PD patients (Levy et al., 2002; Kuhn et al., 2006; Hammond et al., 2007). Consistent with human data, rats with unilateral 6-OHDA lesions to the substantia nigra *pars compacta* (SNc) show increased beta-frequency oscillatory activity in LFPs recorded from the STN, which diminish after administration of the dopamine agonist apomorphine (Sharott et al., 2005; Mallet et al., 2008).

While the literature supports a link between increased power in the beta band frequency range in the cortex and basal ganglia and the development of akinesia/rigidity, beta band activity has not been shown to correlate with the severity of resting tremor (Kuhn et al., 2005; Kuhn et al., 2006; Hammond et al., 2007; Oswal et al., 2013). Rather, the development of tremor in PD patients has been shown to be associated with the emergence of oscillations in the tremor

frequency range (3-7 Hz) in the cortex and basal ganglia (Timmerman et al., 2003; Reck et al., 2009; Hirschmann et al., 2013; Oswal et al., 2013). Timmerman et al., 2003 report a strong coherence between the electromyography (EMG) of forearm muscles and activity in the contralateral primary motor cortex (M1), at tremor (3-7 Hz) and double tremor frequency (7-13 Hz) in PD patients off medication. A similar pattern of activity has been observed in the STN. In African green monkeys treated with MPTP, the development of resting tremor was associated with the emergence of oscillations at tremor frequency and double tremor frequency in the STN (Bergman et al., 1994). Power spectra of STN LFPs in PD patients reveal peaks at tremor frequency and tremor harmonics, as well as significant coherence between STN LFPs and EMG activity at tremor frequency (Brown et al., 2001; Levy et al., 2000; Liu et al., 2002; Wang et al., 2005; Reck et al., 2009). Furthermore, Hirschmann et al. 2013 simultaneously recorded STN LFPs, magnetoencephalography (MEG), and the EMG of forearm muscles in PD patients off medication, and report a positive correlation between tremor-associated increase in STN-cortex coherence and tremor-associated increase in muscle activity. Together, these studies show that cortical and STN power and coherence at tremor frequency increases with the manifestation of tremor.

Present Work

The present set of experiments seeks to characterize aspects of the instigation and treatment of Parkinsonian resting tremor using the tremulous jaw movement model.

Experimental group 1 examines the ability of the galantamine to induce TJMs in mice, as it has yet to be evaluated if administration of an anticholinesterase induces TJMs in mice.

Experimental groups 2 and 3 investigate the antiparkinsonian properties of MAO-B inhibitors by

evaluating behavioral and neurochemical changes. The interaction between dopamine and serotonin neurotransmission that underlies the exacerbation of Parkinsonian motor symptoms after SSRI administration will be addressed in experimental group 4. Finally, experimental group 5 will employ electrophysiological techniques to investigate the neural circuitry that underlies tremorogenesis by examining the relationship between the development of tremor and oscillatory activity in the primary motor cortex and subthalamic nucleus in rats.

Chapter 2: Induction of oral tremor in mice by the acetylcholinesterase inhibitor galantamine: Reversal with adenosine A_{2A} antagonism

2.1 Introduction

Parkinsonism is a family of motor disorders characterized by four cardinal motor symptoms: resting tremor, akinesia, bradykinesia, and rigidity. Idiopathic Parkinson's disease is the most common form of Parkinsonism, while drug administration is the second most common cause (Alvarez et al., 2007; Shulman et al., 2011). Drug-induced Parkinsonism (DIP) can result from administration of pharmacological agents that reduce DA transmission, such as DA antagonists and DA depleting agents (Marsden et al., 1975; McEvoy, 1983; Arbaizar et al., 2008). Thus, DIP has been associated with the administration of a variety of typical and atypical antipsychotics, antiemetics, antihypertensives, gastrointestinal prokinetics, and antivertigo agents (Mena and de Yébenes, 2006; Alvarez et al., 2007; Shin and Chung, 2012). Additionally, several clinical studies report an induction or worsening of parkinsonian motor symptoms after cholinomimetic administration. Anticholinesterases such as tacrine, donepezil, rivastigmine and galantamine are effective in treating cognitive dysfunction in patients with Alzheimer's disease (AD), but have been shown to induce Parkinsonian symptoms, including tremor, as a side effect (Ott and Lannon, 1992; Shea et al., 1998; Arai, 2000; Aarsland et al., 2003; Emre et al., 2004; Litvinenko et al., 2008; Song et al., 2008; Grace et al., 2009; van Laar et al., 2010).

Parkinsonian resting tremor can be modeled in rats using the tremulous jaw movement (TJM) model. TJMs are defined as rapid, repetitive, vertical deflections of the lower jaw that resemble chewing but are not directed at any particular stimulus (Salamone et al., 1998). These movements can be induced by many of the same pharmacological agents that have been shown to produce DIP in humans, including DA antagonists (Steinpreis et al., 1993; Salamone et al.,

1998; Correa et al., 2004; Ishiwari et al., 2005; Salamone et al., 2008a; Betz et al., 2009) and DA depleting agents (Baskin and Salamone, 1993; Salamone and Baskin, 1996; Podurgiel et al., 2013a). Alterations in acetylcholine (ACh) transmission via administration of muscarinic agonists and anticholinesterases also can regulate the production of TJMs (Salamone et al. 1986; Baskin et al., 1994; Salamone et al., 1998; Miwa et al., 2008, 2009; Collins et al., 2010; Collins et al., 2011). In humans, Parkinsonian resting tremor occurs in the frequency range of 3-7 Hz, which is distinct from dyskinesias (1-2 Hz), essential tremor (8 Hz), and postural tremors (8-12 Hz) (Findley et al., 1981, Marsden, 1984; Deuschl et al., 1996, 2000; Collins-Praino et al. 2012). In rats, it also has been reported that TJMs occur predominately in the 3-7.5 Hz frequency range, consistent with the frequency range characteristic of Parkinsonian resting tremor (Salamone et al. 1998; Finn et al., 1998; Cousins et al., 1998; Ishiwari et al., 2005; Collins et al., 2011). Moreover, TJMs in rats can be attenuated using antiparkinsonian agents including L-DOPA (Cousins and Salamone, 1996; Cousins et al. 1998) DA agonists (Baskin and Salamone, 1993; Cousins et al., 1998; Salamone et al., 2005), muscarinic antagonists (Steinpreis et al., 1993; Cousins et al., 1998; Betz et al., 2009), and adenosine A_{2A} antagonists (Correa et al. 2004; Salamone et al., 2008a; Betz et al., 2009; Collins et al., 2010, 2011, 2012; Salamone et al., 2013; Santerre et al., 2012; Podurgiel et al., 2013a).

In order to provide cross-species comparisons, researchers have been expanding the TJM to mice. TJMs in mice were shown to be induced after administration of the muscarinic agonist pilocarpine and the DA-depleting agent tetrabenazine, and both pilocarpine and tetrabenazine-induced TJMs can be attenuated by coadministration of the adenosine A_{2A} antagonist MSX-3 or by conditional neural knockout of the adenosine A_{2A} receptor (Salamone et al., 2013; Podurgiel et al., 2013a). However, it has yet to be determined if administration of an anticholinesterase

induces TJMs in mice. The anticholinesterase galantamine has been reported to induce or worsen tremor in human patients (Aarsland et al., 2003; Litvinenko et al., 2008; Grace et al., 2009) and induces TJMs in rats (Collins et al., 2011; Podurgiel et al., 2013b). The present study sought to develop a mouse model of cholinomimetic-induced TJMs using the anticholinesterase galantamine. The first experiment examined the ability of galantamine to induce TJMs in mice, and the second experiment assessed the ability of the adenosine A_{2A} antagonist MSX-3 to attenuate galantamine-induced TJMs. The final experiment employed freeze-frame video analyses to evaluate the local frequency of TJMs induced by galantamine, as well as the tremorolytic effects of MSX-3. This experiment was conducted to determine if galantamine-induced TJMs would occur predominantly in the 3-7.5 Hz frequency range, and also if co-administration of MSX-3 would alter the local frequency characteristics of the of galantamine-induced TJMs.

2.2 Materials and Methods

Animals

Male C57BL/6J mice (N=37) weighed 35-45 g during the course of the experiment and had ad libitum access to lab chow and water (Jackson Laboratories, Bar Harbor, ME). They were group-housed in a colony that was maintained at approximately 23°C and had a 12-hour light/dark cycle (lights on at 0700 hrs). These studies were conducted according to University of Connecticut and NIH guidelines for animal care and use.

Drug Treatment Procedures and Dose Selection

The acetylcholinesterase inhibitor galantamine hydrobromide ((4a*S*,6*R*,8a*S*) 5,6,9,10,11,12-hexahydro-3-methoxy-11-methyl-4a*H*-[1]benzofuro[3*a*,3,2-*ef*] [2] benzazepin-6-ol) was obtained from Tocris Bioscience (Bristol, UK). Galantamine was dissolved in 0.9% saline. MSX-3 ((*E*)-phosphoric acid mono-[3-[8-[2-(3-methoxyphenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl] ester), the adenosine A_{2A} antagonist, was synthesized at the Pharmazeutisches Institut Universität Bonn; Bonn, Germany (see Hockemeyer et al., 2004). MSX-3 was dissolved in 0.9% saline. The pH of the MSX-3 solution was adjusted by adding 1.0 N NaOH until the drug was completely in solution after conversion to its disodium salt (pH 7.1 – 7.7). MSX-3 is a prodrug that is converted into the active adenosine A_{2A} antagonist, MSX-2, *in vivo*. The doses of galantamine (0.5, 1.0, 1.5, 2.0, 2.5 mg/kg) were selected based on extensive pilot studies, and doses of MSX-3 (2.5, 5.0, 10.0 mg/kg) were chosen based on previous data from our laboratory (Salamone et al., 2013).

Behavioral Procedure: Tremulous Jaw Movements

Observations of mice took place in a 11.5 × 9.5 × 7.5 cm clear glass chamber with a wire mesh floor, which was elevated 26 cm from the table top. Tremulous jaw movements were defined as rapid vertical deflections of the lower jaw that resembled chewing but were not directed at any particular stimulus. Each individual deflection of the jaw was recorded using a mechanical hand counter by a trained observer, who was blind to the experimental condition of the mouse being observed. Separate studies with two observers demonstrated an inter-rater reliability of $r=0.98$ ($p<0.001$) using these methods in mice.

Experiments

Experiment 1: Ability of galantamine to induce tremulous jaw movements in mice

A group of 15 mice was used to assess the ability of galantamine to induce TJMs. All mice received IP injections of either 1.0 ml/kg saline (vehicle) or 0.5, 1.0, 1.5, 2.0, or 2.5 mg/kg galantamine in a within-groups design, with all mice receiving all drug treatments in a randomly varied order (one treatment per week; no treatment sequences repeated). Twenty minutes after IP injection, the mice were placed in the chamber and allowed to habituate for five minutes. Following the habituation period, TJMs were counted for fifteen minutes, with the observation period divided into three five-minute epochs.

Experiment 2: Ability of the adenosine A_{2A} antagonist MSX-3 to attenuate tremulous jaw movements induced by galantamine

A group of 10 mice was used to assess the effect of administration of the adenosine A_{2A} antagonist MSX-3 on TJMs induced by 2.5 mg/kg galantamine. A within-groups design was utilized for this study, with all mice receiving all drug treatments in a randomly varied order (one treatment per week; no treatment sequences repeated). On the test day each week, each mouse received an IP injection of either 1.0 ml/kg saline (vehicle) or 2.5 mg/kg galantamine. After 5 minutes, mice received an IP injection of either 1.0 ml/kg saline (vehicle) or 2.5, 5.0, or 10.0 mg/kg MSX-3. Fifteen minutes after the second injection, mice were placed in the observation chamber and allowed to habituate for 5 minutes. TJMs were then counted for 15 minutes, with the observation period divided into three five-minute epochs.

Experiment 3: Freeze-frame video analysis of the local frequency of tremulous jaw movements induced by galantamine

A total of 12 mice were used to examine the local frequency of galantamine-induced TJMs and the local frequency of TJMs after coadministration of galantamine and MSX-3. A within-groups design was utilized for this study, with all mice receiving all drug treatments in a randomly varied order (one treatment per week; no treatment sequences repeated). On the test day each week, each mouse received an IP injection of 2.5 mg/kg galantamine. After 5 minutes, mice received an IP injection of either 1.0 ml/kg saline (vehicle) or 10.0 mg/kg MSX-3. After 15 minutes, mice were placed in a flat bottomed mouse restrainer (myNeuroLab.com, Richmond, IL) so that a consistent view of the orofacial area could be achieved. After habituating for 5 minutes, each mouse was recorded for 15 minutes (using a Cannon PowerShot SX120 digital camera, in video mode.) The sections of video were subjected to a freeze-frame analysis (1 frame=1/30 s), in which the observer went frame-by-frame through each burst of jaw movements (i.e. each group of at least two jaw movements that were within 1.0 s of each other). The observer recorded the inter-movement interval for each pair of jaw movements within bursts, which was defined as the number of frames between each point at which the jaw was fully open during successive jaw movements. This information was used to determine the local frequency within bursts of jaw movements.

Data Analyses

The data for experiments 1 and 2 were analyzed using a repeated measures analysis of variance (ANOVA). A computerized statistical program (SPSS 21.0 for Windows) was used to perform these analyses. Average TJMs per five-minute observation period were calculated and

used in the ANOVA calculations. When there was a significant ANOVA in experiments 1 and 2, planned comparisons using the overall error term were used to assess the differences between each dose and the control condition (the number of comparisons was restricted to the number of treatments minus one; Keppel, 1991). The video files from experiment 3 were analyzed using Adobe Premiere CS5.5 for Windows, which allowed freeze-frame analysis in a frame-by-frame sequence (1 frame = 1/30 s). The data for experiment 3 were analyzed by calculating a relative frequency distribution (i.e., each bin represented as percent of total inter movement intervals), and the interaction term from the repeated measures factorial ANOVA was used to determine if the relative distributions of inter-movement times differed between the galantamine and the galantamine plus MSX-3 conditions.

2.3 Results

Experiment 1: Ability of galantamine to induce tremulous jaw movements in mice

Repeated measures ANOVA revealed a significant overall effect of galantamine on TJMs (Figure 2.1; $F(5,70) = 4.271$; $p < 0.05$). The 1.5, 2.0, and 2.5 mg/kg dose of galantamine significantly induced TJMs (planned comparisons, $p < 0.05$) compared to vehicle control.

Experiment 2: Ability of the adenosine A_{2A} antagonist MSX-3 to attenuate tremulous jaw movements induced by galantamine

As shown in Figure 2.2, there was a significant overall effect of MSX-3 co-administration on galantamine-induced TJMs ($F(4,36) = 6.187$; $p < 0.05$). The 2.5 mg/kg dose of galantamine significantly induced TJMs compared to vehicle control (planned comparisons,

$p < 0.05$), and all doses of MSX-3 (2.5, 5.0, and 10.0 mg/kg) significantly attenuated galantamine-induced TJMs (planned comparisons, $p < 0.05$).

Experiment 3: Freeze-frame video analysis of the local frequency of tremulous jaw movements induced by galantamine

Analysis of the videos showed that there were 48.2 ± 6.5 identified TJMs shown by rats tested in the galantamine plus vehicle condition, and 31.8 ± 7.9 in the galantamine plus MSX-3 condition. Analysis with the matched-pair t-test revealed that MSX-3 significantly reduced the number of TJMs in galantamine-treated mice relative to galantamine alone ($t = 2.97$, $df = 4$, $p < 0.05$). Figure 2.3 shows the relative frequency distributions of inter movement times for the galantamine plus saline and galantamine plus MSX-3 conditions. Repeated measures factorial ANOVA revealed that there was a significant overall difference across inter-movement interval bins ($F(29,232) = 67.7$; $p < 0.0001$) and a significant interval bin x drug condition interaction ($F(29,232) = 1.58$; $p < 0.05$). The significant interaction indicates that the relative distribution of inter-movement intervals in galantamine-treated rats was altered by co-administration of MSX-3. Four of five rats treated with galantamine plus saline showed a peak number of inter-movement intervals in bin 5, while four of five rats treated with galantamine plus MSX-3 showed a peak in bin 6.

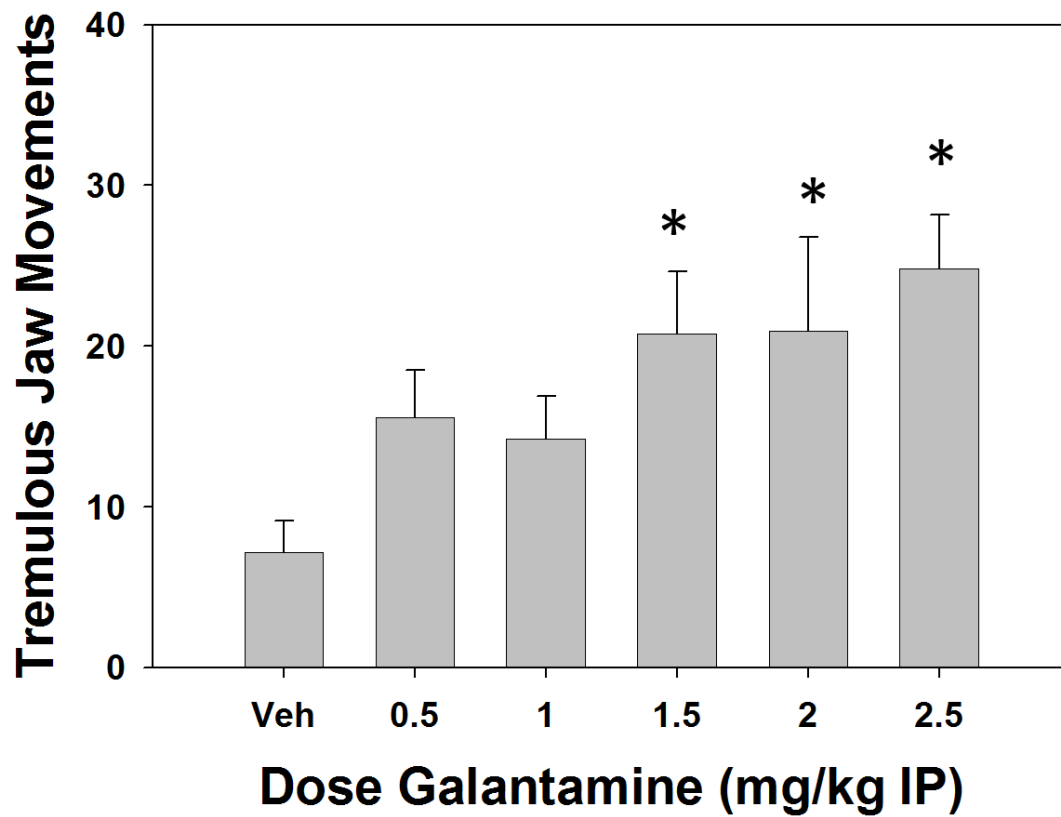


Figure 2.1: Effects of different doses of galantamine (IP) on tremulous jaw movements. Mean (\pm SEM) number of jaw movements (per 5 min period) in mice treated with either saline vehicle or galantamine. *significant difference from vehicle control ($p < 0.05$)

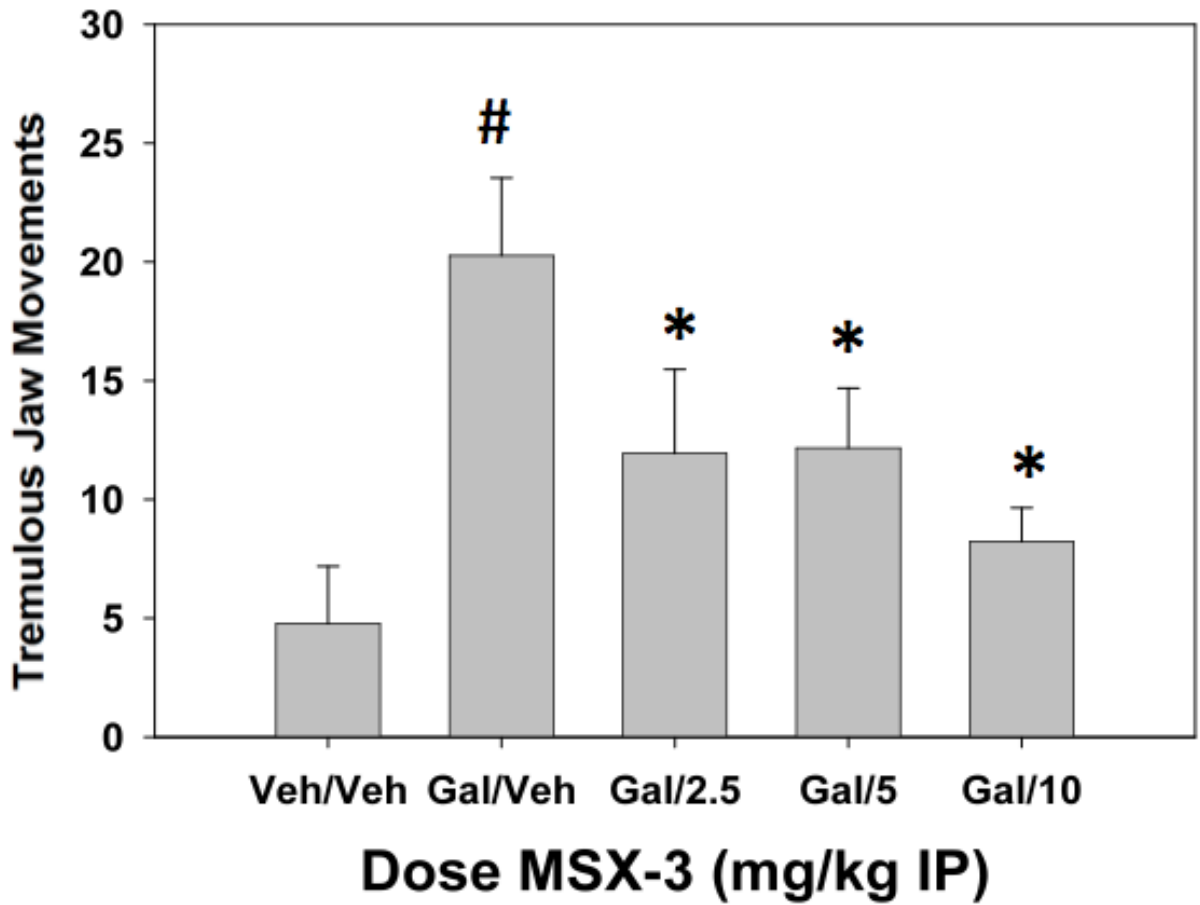


Figure 2.2: Effect of adenosine A_{2A} antagonism on galantamine (2.5 mg/kg) induced TJMs. Mean (\pm SEM) number of jaw movements (per 5 min period) in mice treated with vehicle, galantamine plus vehicle (Gal/Veh), and galantamine plus various doses (2.5-10.0 mg/kg) of MSX-3. # significant difference from vehicle; *significant difference from galantamine plus vehicle control ($p < 0.05$)

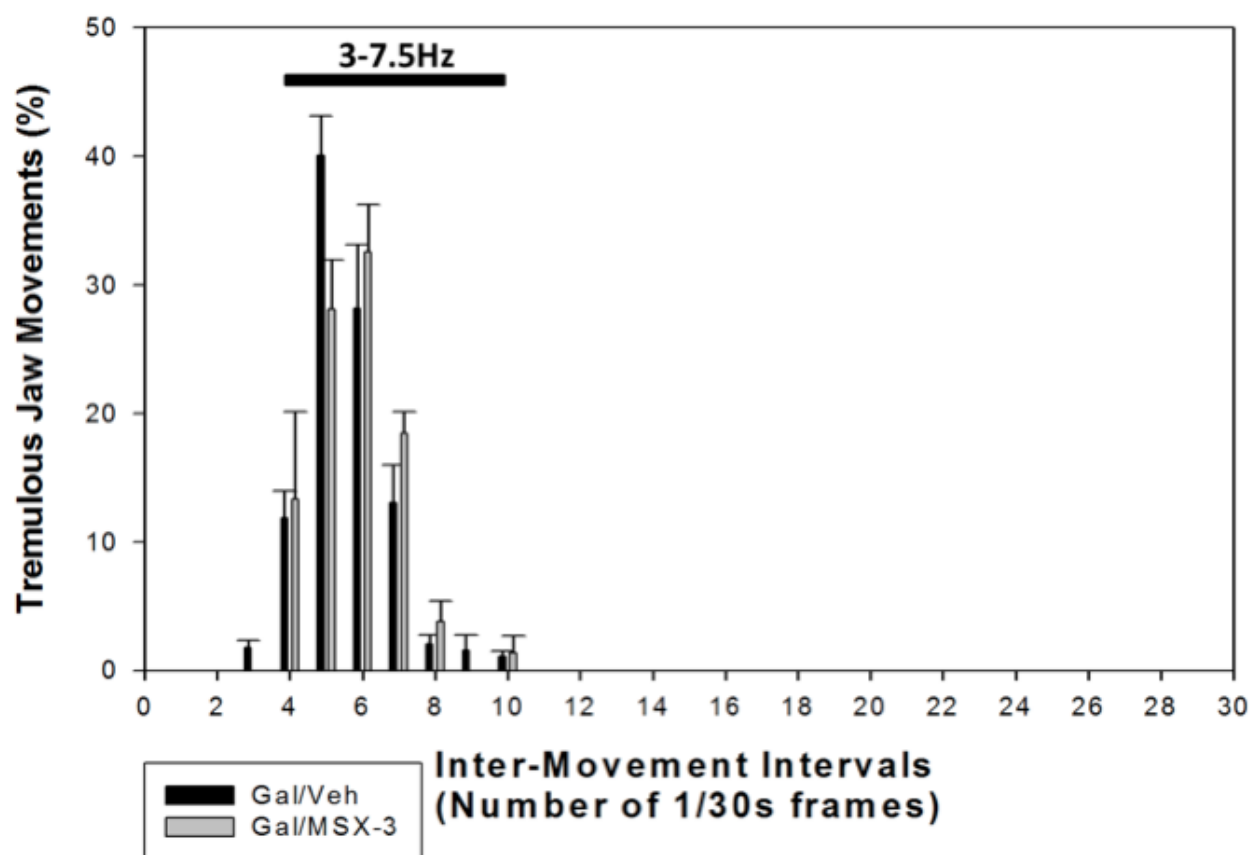


Figure 2.3: Results of the freeze-frame video analysis, showing the relative frequency distribution of inter-movement intervals across several time bins. The mean (\pm SEM) number of inter-movement intervals, expressed as a percent of total, is shown for each frequency bin, for mice treated with galantamine plus saline vs. galantamine plus MSX-3. There was a significant interval bin x drug treatment interaction (see text for details).

Chapter 3: Tremorolytic effects of safinamide in animal models of drug induced parkinsonian tremor

Published; Podurgiel et al., 2013

3.1 Introduction

In idiopathic Parkinson's disease, neurodegenerative processes that deplete striatal dopamine (DA) result in the development of motor symptoms including akinesia, bradykinesia, rigidity and tremor (Bernheimer et al., 1973). In addition, several classes of drugs are known to induce parkinsonian symptoms in humans. Administration of antipsychotic drugs that block DA receptors (e.g., haloperidol, chlorpromazine, pimozide) or deplete striatal DA (e.g. reserpine or tetrabenazine) has been shown to induce parkinsonian motor symptoms (Marsden et al., 1975; McEvoy, 1983; Arbaizar et al., 2008). Furthermore, cholinomimetic drugs are known to be tremorogenic (Brimblecomb, 1975; Dronfield et al. 2000; Liston et al. 2004; Salamone et al. 2001), and several clinical studies have reported that cholinomimetics can induce or exacerbate parkinsonian symptoms, including tremor, in humans (Aarsland et al. 2003; Arai, 2000; Bourke and Drukenbrod, 1998; Cabeza-Alvarez et al. 1999; Duvoisin, 1967; Gurevich et al. 2006; Iwasaki et al. 1988; Kao et al. 1993; Keltner, 1994; McSwain and Forman, 1995; Ott and Lannon, 1992; Shea et al. 1998; Song et al. 2008). The most common treatment for idiopathic Parkinson's disease is the DA precursor L-DOPA, but parkinsonian symptoms also are treated by a number of other dopaminergic and non-dopaminergic agents, including DA agonists, muscarinic acetylcholine antagonists (Aquilonius, 1980; McEvoy, 1983), and amantadine. Novel therapeutic approaches include adenosine A_{2A} antagonists, gene therapies, and various surgical procedures intended to restore neurochemical balance in the basal ganglia circuitry (Salamone 2010; Hauber 2011).

Monoamine oxidase B (MAO-B) inhibitors have also been employed as a treatment for parkinsonism (Moussa et al. 2006; Onofrj et al. 2008; Schapira 2010). MAO-B is one of the key enzymes responsible for dopamine metabolism in the brain (Moussa et al. 2006). Selegiline and rasagiline are potent, irreversible MAO-B selective inhibitors that are presently available for the treatment of PD (Onofrj et al. 2008; Schapira 2010). Safinamide, ((S)-(+)-2-[4-(3-fluorobenzyloxybenzylamino)pro-panamide]methanesulfonate (1:1 salt), is a water soluble, α -aminoamide derivative with multiple actions (Marzo et al. 2004; Fariello 2007; Onofrj et al. 2008; Schapira 2010; Stocchi 2012). Safinamide is a potent, highly selective and reversible inhibitor of MAO-B (Marzo et al. 2004; Fariello 2007; Onofrj et al. 2008; Schapira 2010; Stocchi 2012). Additionally, safinamide reduces dopamine uptake, blocks voltage-dependent sodium channels, modulates N-type calcium channels, and reduces glutamate release (Marzo et al. 2004; Fariello 2007; Onofrj et al. 2008; Schapira 2010; Stocchi 2012). By doing so, animal studies have revealed that safinamide exerts neuroprotectant, anticonvulsant, and antiparkinsonian properties (Salvati et al. 1999; Fariello et al. 2000; Gregoire et al. 2010). Safinamide has an excellent therapeutic and safety margin and is currently in Phase III clinical trial development as an add-on therapy to levodopa or dopamine agonists for PD patients (Marzo et al. 2004; Onofrj et al. 2008; Schapira 2010; Stocchi 2012).

Several animal models have been used to assess various motor functions related to parkinsonism (Avila et al. 2009; Castañeda et al. 2005; Pollack and Thomas, 2009). Although resting tremor is one of the cardinal symptoms of parkinsonism, relatively little information is known about the neural mechanisms underlying tremorogenesis or its treatment (Bergman and Deuschl et al. 2002; Deuschl et al. 2001; Fishman 2008; Binder et al. 2009), and research employing animal models of tremor also can contribute greatly to our understanding of the

neurochemical regulation of tremorogenesis (Miwa 2007; Salamone et al. 1998; Wilms et al. 1999). For this reason, the present studies used the tremulous jaw movement model, which is a rodent model of parkinsonian tremor that has been extensively employed (Cenci et al. 2002; Cousins and Salamone, 1998; Ishiwari et al. 2005; Miwa et al. 2008, 2009; Rodriguez-Diaz et al. 2001; Salamone et al. 1990, 1998, 2001, 2005, 2008a, 2008b; Simola et al. 2004, 2006; Vanover et al. 2008). These movements are defined as repetitive vertical deflections of the lower jaw that resemble chewing but are not directed at a particular stimulus (Salamone et al. 1998). As shown by studies using videotape analyses or electromyographic methods, these movements occur largely within the 3-7 Hz frequency range that is characteristic of parkinsonian resting tremor (Cousins et al. 1998; Finn et al. 1997; Ishiwari et al. 2005; Mayorga et al. 1997), and can be induced by a number of conditions that parallel the neurochemistry of the pathology of parkinsonism, including striatal DA depletion, DA antagonism, anticholinesterases and muscarinic agonists (Baskin and Salamone, 1993; Betz et al. 2005; Cousins and Salamone 1998; Finn et al. 1997; Ishiwari et al. 2005; Jicha and Salamone 1991; Mayorga et al. 1997; Rodriguez-Diaz et al. 2001; Salamone and Baskin, 1996; Salamone et al. 1990, 1998, 2005, 2008a; Steinpreis et al. 1993; Trevitt et al. 1998). Dopaminergic antiparkinsonian drugs such as apomorphine, L-DOPA, bromocriptine, pergolide, and ropinirole can reduce cholinomimetic-induced tremulous jaw movements (Cousins et al., 1997; Salamone et al., 2005), and their potency for suppressing cholinomimetic-induced tremulous jaw movements is highly correlated ($r = 0.88$) with the clinical potency of these drugs for reducing parkinsonian tremor in humans (Salamone et al., 2005). Tremulous jaw movements are sensitive to several other classes of antiparkinsonian drugs, including muscarinic antagonists and adenosine A_{2A} antagonists (Baskin

and Salamone 1993; Betz et al. 2007, 2009; Correa et al. 2004; Cousins et al. 1997; Salamone et al. 1998, 2008a; Simola et al. 2004, 2006; Steinpreis et al. 1993; Tronci et al. 2007).

The present experiments examined the ability of safinamide to attenuate drug-induced tremulous jaw movements in rats. Because cholinomimetics are well known tremorogenic agents (Brimblecombe 1975; Salamone et al. 2001; Collins-Praino et al. 2011), the first two experiments will employ cholinomimetics that are known to induce tremulous jaw movements in order to assess the effects of safinamide. The first experiment assessed the ability of safinamide to reverse tremulous jaw movements induced by the anticholinesterase galantamine (Collins et al. 2011). The second experiment examined the ability of safinamide to attenuate tremulous jaw movements induced by the muscarinic agonist pilocarpine (Collins et al. 2010a). The final experiment studied the ability of safinamide to reverse the effects of the DA D2 antagonist pimozide on tremulous jaw movements and locomotor suppression, under the same conditions used previously for the assessment of other antiparkinsonian agents (Salamone et al. 2008a). These conditions (i.e., repeated administration of 1.0 mg/kg pimozide) were optimized for induction of tremulous jaw movements (Ishiwari et al. 2005; Salamone et al. 2008a), but also allow for assessment of locomotion. It was hypothesized that safinamide would be able to reverse the tremorogenic effects of galantamine, pilocarpine, and pimozide.

3.2 Materials and Methods

Animals

A total of 118 adult male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) with no prior drug experience were used in the present experiments. The rats weighed 350-450 g during the course of the experiment and had *ad libitum* access to lab chow and water. They

were group-housed in a colony that was maintained at approximately 23°C and had a 12-hour light/dark cycle (lights on at 0700 hrs). These studies were conducted according to University of Connecticut and NIH guidelines for animal care and use.

Drug Treatment Procedures and Dose Selection

Galantamine hydrobromide was obtained from Tocris Bioscience (Bristol, UK) and dissolved in 0.9% saline. Pilocarpine and pimoziide were purchased from Sigma Aldrich Chemical (St. Louis, MO). Pilocarpine was dissolved in 0.9% saline, and pimoziide was dissolved in a 0.3% tartaric acid solution (final pH = 4.0). Safinamide, (S)-(+)-2-[4-(3-fluorobenzyloxybenzylamino)pro-panamide]methanesulfonate (1:1 salt) is a water soluble, α -aminoamide derivative. Safinamide was obtained from Merck Serono International S.A. (Geneva, Switzerland) and was dissolved in 0.9% saline, which was also used as the vehicle control. An acute dose of 3.0 mg/kg (IP) galantamine or 0.5 mg/kg (IP) pilocarpine was used for the studies examining galantamine or pilocarpine-induced tremulous jaw movements. The selection of these doses was based on previously published experiments showing induction of jaw movements at these doses (Collins et al. 2010a, Collins-Praino et al. 2011a). Subchronic 1.0 mg/kg (IP) pimoziide treatment was used for the study examining tremulous jaw movements and locomotion. This treatment procedure was based upon previously published experiments showing induction of jaw movements at this dose (Ishiwari et al. 2005; Betz et al., 2007, 2009; Salamone et al. 2008a; Collins et al. 2010b). Subchronic 1.0 mg/kg (IP) pimoziide treatment is optimized for the production of jaw movement activity and also allows for the parallel assessment of locomotion. The procedure of screening animals by assessing them for tremulous jaw movements the day before the drug challenge day was the same as that used in previous

studies (Ishiwari et al. 2005; Salamone et al. 2008a; Collins et al. 2010b). This was done in order to ensure a robust jaw movement response on the drug challenge day. None of the animals failed to show a substantial jaw movement response to pimozide on day 7. The doses of safinamide chosen were based upon extensive pilot work.

Behavioral Procedures

Tremulous jaw movements. Observations of rats took place in a 30 × 30 × 30 cm clear Plexiglas chamber with a wire mesh floor, which was elevated 42 cm from the table top. This allowed for the viewing of the animal from several angles, including underneath. Tremulous jaw movements were defined as rapid vertical deflections of the lower jaw that resembled chewing but were not directed at any particular stimulus (Salamone et al. 1998). Each individual deflection of the jaw was recorded using a mechanical hand counter by a trained observer, who was blind to the experimental condition of the rat being observed.

Locomotor activity. Locomotor activity was assessed by placing the rat into an automated activity chamber (28 cm X 28cm X 28 cm) enclosed in a sound-attenuating shell. The floor of the chamber was elevated 6 cm above the chamber bottom and was composed of two moveable wire-mesh panels, (25 cm x 12 cm), which were further divided into four quadrants by means of a central metal rod between the two panels. As the rat entered each quadrant, a slight vertical movement of the mesh panels closed a microswitch located outside of the locomotion chamber. This depression was detected and recorded by a computer program, written in MedPC, as a single activity count (Med Associates, Inc., Georgia, VT). The locomotor activity session was 30-min in length. These methods of measuring locomotion have been used previously to assess the effects of DA and antagonists on locomotion (Collins et al. 2010a; Salamone et al., 2008a).

Experiments

Experiment 1: Ability of safinamide to reverse tremulous jaw movements induced by the anticholinesterase galantamine.

A group of 7 rats was used to assess the effects of safinamide on the tremulous jaw movements induced by administration of 3.0 mg/kg galantamine. A within-groups design was used for this study, with all rats receiving all drug treatments in a randomly varied order (one treatment per week; no treatment sequences were repeated). On test day each week, all rats received an IP injection of 3.0 mg/kg galantamine 30 min before the test session. Concurrently, each rat was given an IP injection of either 1.0 mL/kg saline or 0.312 mg/kg, 0.625 mg/kg, 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg, or 10.0 mg/kg safinamide (30 min before the test session). Twenty min after injections, rats were placed in the Plexiglas observation chamber and allowed 10 min to habituate, after which tremulous jaw movements were counted for 15 min, following the same procedure outlined above.

Experiment 2: Ability of safinamide to reverse tremulous jaw movements induced by the muscarinic agonist pilocarpine.

A group of 14 rats was used to assess the effects of safinamide on the tremulous jaw movements induced by administration of 0.5 mg/kg pilocarpine. A within-groups design was used for this study, with all rats receiving all drug treatments in a randomly varied order (one treatment per week; no treatment sequences were repeated). On test day each week, all rats received an IP injection of 0.5 mg/kg pilocarpine 10 min before the test session. In addition, each rat received an IP injection of either 1.0 mL/kg saline or 0.312 mg/kg, 0.625 mg/kg, 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg, or 10.0 mg/kg safinamide 30 min before the test session. Immediately

after pilocarpine injections, rats were placed in the Plexiglas observation chamber and allowed 10 min to habituate, after which tremulous jaw movements were counted for 15 min, following the same procedure outlined above.

Experiment 3: Ability of safinamide to reverse tremulous jaw movements and locomotor suppression induced by subchronic administration of the DA D2 antagonist pimozide.

A group of 97 rats was used to assess the effects of subchronic systemic injections of the DA D2 antagonist pimozide on tremulous jaw movements. One group of rats received repeated daily injections of vehicle, and also received vehicle injections on the test day. All other rats received an injection of 1.0 mg/kg pimozide IP for 8 consecutive days. On day 7 of the subchronic injections, rats were assessed for the induction of tremulous jaw movements in a five min period. Any rat that showed less than 15 tremulous jaw movements on day 7 was excluded from further testing. Three hrs and 30 min following their daily pimozide injection on day 8, rats received an IP injection of vehicle control or safinamide (saline vehicle control, 0.625 mg/kg, 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg, 10.0 mg/kg safinamide). Twenty minutes later, animals were placed in the Plexiglas observation chamber and allowed to habituate for 10 min. After the habituation period, the number of jaw movements in a 15-min observation period was assessed as described above. Immediately after completion of the tremulous jaw movement test, locomotor activity was assessed in a 30-min session, using the procedure outlined above.

Data Analyses

Behavioral data for experiments 1 and 2 was analyzed using a repeated measures analysis of variance (ANOVA). Behavioral data for experiment 3 was analyzed using a between groups

analysis of variance (ANOVA). A computerized statistical program (SPSS 19.0 for Windows) was used to perform all analyses. When there was a significant ANOVA, planned comparisons using the overall error term were used to assess the differences between each dose and the control condition; the total number of comparisons was restricted to the number of treatments minus one (Keppel, 1991).

3.3 Results

Experiment 1: Ability of safinamide to reverse tremulous jaw movements induced by the anticholinesterase galantamine.

Figure 3.1 shows the effects of systemic injections of safinamide on tremulous jaw movements induced by galantamine. Co-administration of safinamide produced a significant reduction in galantamine induced tremulous jaw movements. ANOVA revealed a significant overall treatment effect ($F(6,36) = 3.54, p < 0.05$). Planned comparisons revealed that each dose of safinamide plus galantamine differed significantly from galantamine plus vehicle ($p < 0.05$).

Experiment 2: Ability of safinamide to reverse tremulous jaw movements induced by the muscarinic agonist pilocarpine

Figure 3.2 shows the effects of systemic injections of safinamide on tremulous jaw movements induced by pilocarpine. Co-administration of safinamide produced a significant reduction in pilocarpine induced tremulous jaw movements. ANOVA revealed a significant overall treatment effect ($F(6,78) = 2.51, p < 0.05$). Planned comparisons revealed that three doses of safinamide (0.625 mg/kg, 5.0 mg/kg, 10.0 mg/kg) plus pilocarpine differed significantly from pilocarpine plus vehicle ($p < 0.05$).

Experiment 3: Ability of safinamide to reverse tremulous jaw movements and locomotor suppression induced by subchronic administration of the DA D2 antagonist pimozide.

Figure 3.3 shows the effects of systemic injections of safinamide on tremulous jaw movements induced by pimozide. Co-administration of safinamide produced a significant reduction in pimozide induced tremulous jaw movements. ANOVA revealed a significant overall treatment effect ($F(6,90) = 7.6, p < 0.05$; pimozide significantly induced tremulous jaw movements relative to Veh/Veh ($p < 0.05$). Planned comparisons revealed that three doses of safinamide (1.25 mg/kg, 5.0 mg/kg, 10.0 mg/kg) plus pimozide differed significantly from pimozide plus vehicle ($p < 0.05$). There was an overall significant drug treatment effect ($F(6,87) = 20.3, p < 0.001$). Pimozide significantly reduced locomotor activity counts relative to Veh/Veh ($p < 0.05$). However, there were no significant effects of safinamide on pimozide-induced suppression of locomotion.

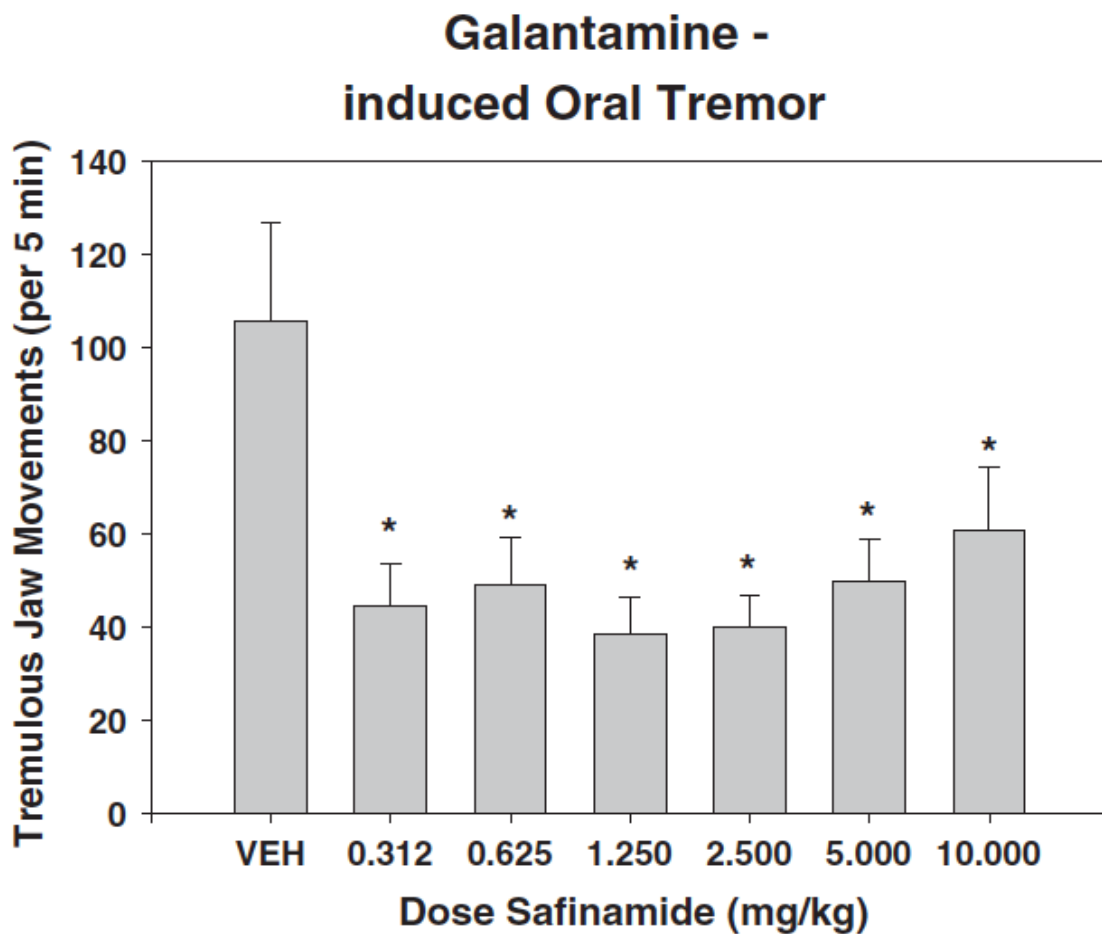


Figure 3.1: Effect of safinamide on the tremulous jaw movements induced by 3.0 mg/kg of the acetylcholinesterase inhibitor galantamine. Mean (\pm SEM) number of jaw movements in rats treated with galantamine plus vehicle (Veh), and galantamine plus various doses (0.3125-10.0 mg/kg IP) of safinamide. *significant difference from galantamine plus vehicle (Veh) control ($p < 0.05$)

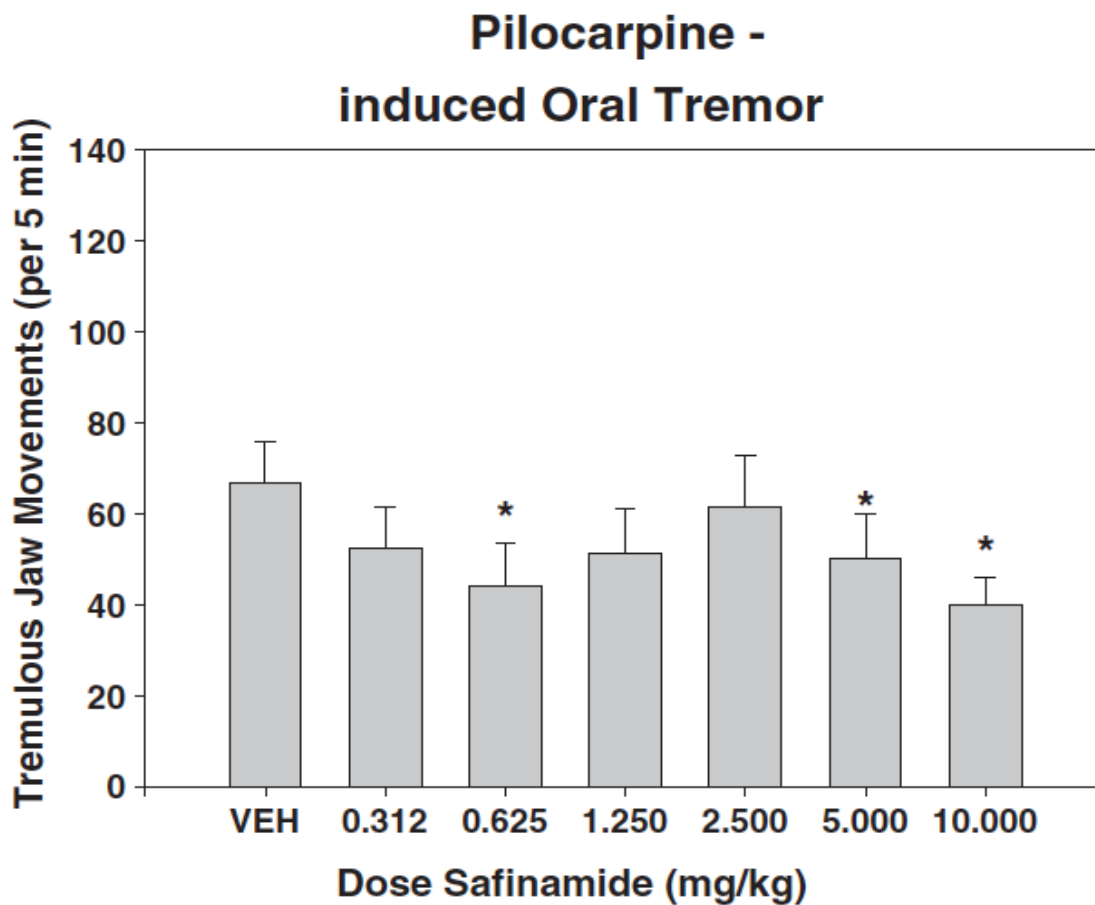


Figure 3.2: Effect of safinamide on the tremulous jaw movements induced by 0.5 mg/kg of the muscarinic acetylcholine receptor agonist pilocarpine. Mean (\pm SEM) number of jaw movements in rats treated with pilocarpine plus vehicle (Veh), and pilocarpine plus various doses (0.3125-10.0 mg/kg IP) of safinamide. *significant difference from pilocarpine plus vehicle (Veh) control ($p < 0.05$)

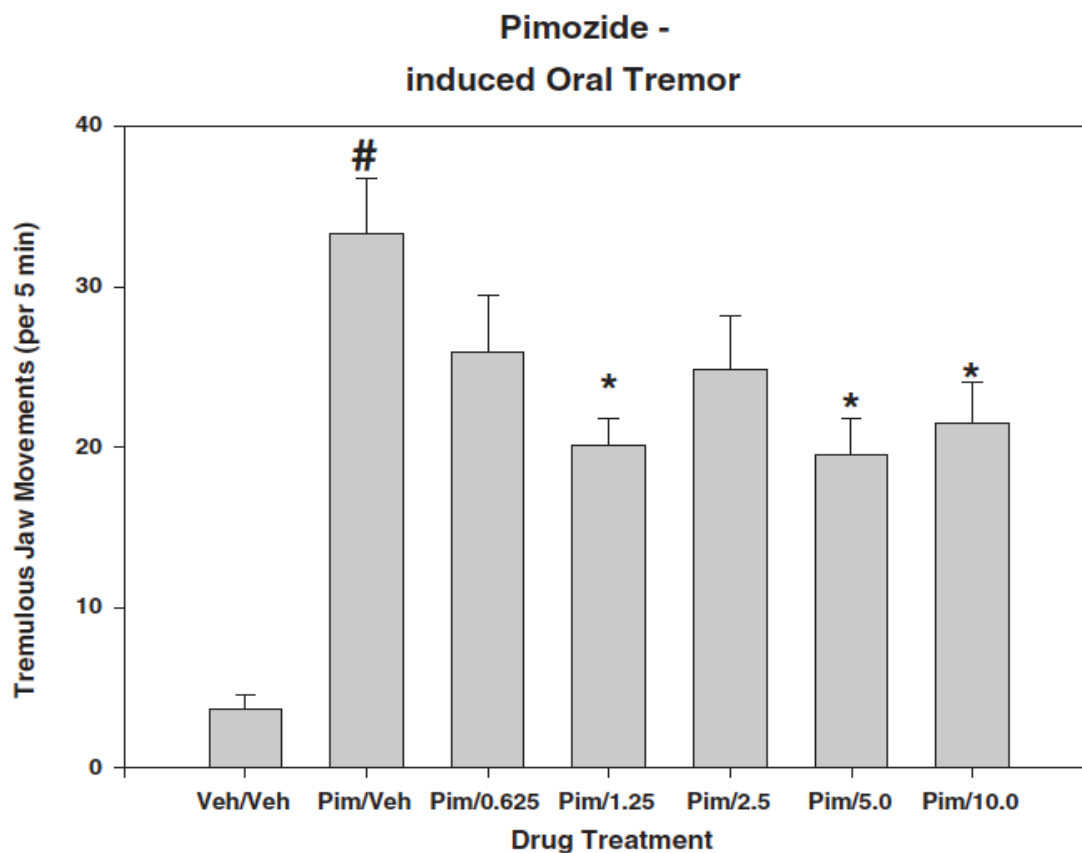


Figure 3.3: Effect of safinamide on pimozide-induced tremulous jaw movements. Mean (\pm SEM) number of individual jaw movements (per 5 min observation period) after injection of tartaric acid vehicle plus 1.0 mg/kg pimozide or pimozide plus various IP doses of safinamide.

* $p < 0.05$, different from vehicle plus pimozide.

Chapter 4: MAO-B inhibition attenuates Parkinsonism induced by the VMAT-2 inhibitor tetrabenazine

4.1 Introduction

Idiopathic Parkinson's disease (PD) results from the death of dopamine (DA) producing cell bodies in the substantia nigra *pars compacta*, which causes the degeneration of the nigrostriatal pathway and depletion of DA in striatal areas (Hornykiwicz, 1973). Idiopathic PD is one member of a broader family of motor disorders known as Parkinsonism, the members of which share five cardinal motor symptoms: resting tremor, akinesia, bradykinesia, rigidity, and postural instability (Marsden et al, 1975; Ostrem and Galifianakis, 2010). While idiopathic PD is the most common cause of Parkinsonism, administration of pharmacological agents that decrease DA or increase acetylcholine (ACh) neurotransmission is the second most common cause (Ostrem and Galifianakis, 2010). Rodent models are a valuable tool for studying Parkinsonism, and several behavioral paradigms exist to evaluate the motor symptoms. Catalepsy and locomotor activity can be used to model symptoms such as akinesia and bradykinesia, and the tremulous jaw movement (TJM) model is widely used for studying parkinsonian tremor. TJMs, defined as "vertical deflections of the lower jaw that resemble chewing but are not directed at any particular stimulus" (Salamone et al., 1998) are induced in rats by many of the same pharmacological agents that are responsible for drug-induced Parkinsonism in humans, including dopamine (DA) depleting agents (Baskin and Salamone, 1993; Podurgiel et al., 2013a), DA antagonists (Steinpreis et al., 1993; Ishiwari et al., 2005) and cholinomimetics (Salamone et al. 1986; Collins et al., 2011). TJMs have a local frequency of 3-7.5 Hz, which parallels the frequency range characteristic of resting tremor in humans (Salamone et al. 1996, 1998; Finn et al., 1997; Mayora et al., 1997; Cousins et al., 1998; Ishiwari et al., 2005; Collins et al., 2011),

and can be attenuated by coadministration of antiparkinsonian agents (Baskin and Salamone, 1993; Steinpreis et al., 1993; Cousins and Salamone, 1996; Cousins et al. 1997; Mayorga et al., 1997; Correa et al. 2004; Salamone et al., 2005; Betz et al., 2009, Salamone et al., 2008; Collins et al., 2010a, 2011, 2012; Santerre et al., 2012; Podurgiel et al., 2013a, 2013b).

Tetrabenazine (TBZ) is a reversible and selective inhibitor of the type-2 vesicular monoamine transporter (VMAT-2; Erickson et al., 1996), which was approved by the Food and Drug Administration in 2008 for treatment of chorea associated with Huntington's disease (HD) (de Tommaso et al., 2011). TBZ blocks vesicular storage and depletes monoamines, but has its greatest effects upon DA neurons, and exhibits its highest binding density in DA-rich areas of the brain including the caudate/putamen, substantia nigra *pars compacta*, and nucleus accumbens (Pettibone et al., 1984a, 1984b; Pearson and Reynolds, 1988; Thibaut et al., 1995; Tanra et al., 1995; German et al., 2000). Research with humans has indicated that TBZ is effective at treating HD-associated chorea in both the short and long-term (Kenney et al., 2007a,b; Frank, 2009; Poon et al., 2010; de Tommaso et al., 2011; Chen et al., 2012), but also can induce adverse events, including drowsiness and depression, as well as Parkinsonian motor symptoms (Kenney et al., 2007a; Frank, 2009).

Our laboratory has recently established a pharmacological rodent model of Parkinsonism using TBZ (Podurgiel et al., 2013a). When administered to rats, TBZ induces TJMs, catalepsy, and locomotor suppression, and coadministration of the adenosine A_{2A} antagonist MSX-3, a putative antiparkinsonian agent, attenuates these motor deficits (Podurgiel et al., 2013a). Furthermore, MSX-3 reverses TBZ-induced c-Fos expression in the ventrolateral striatum (VLS) (Podurgiel et al., 2013a), the striatal subregion associated with the production TJMs (Salamone et al., 1990; Jicha and Salamone, 1991). Since it is critical to validate animal models, and MSX-3

has been the only antiparkinsonian agent used to attenuate TBZ-induced Parkinsonism, this study examined the behavioral and neurochemical effects of administration of the well-established antiparkinsonian agent deprenyl (Selegiline) on TBZ-induced Parkinsonism. Deprenyl is a potent, irreversible, selective inhibitor of monoamine oxidase type B (MAO-B), one of the key enzymes responsible for dopamine metabolism in the brain (Youdim and Bakhle, 2006). Deprenyl has been employed for over 30 years for the treatment of motor symptoms in early and late stage PD (Schapira et al., 2011; Riederer P, Laux G, 2011; Fabbrini et al., 2012). The present study examined the ability of deprenyl to attenuate TBZ-induced TJMs, and assessed extracellular DA levels in the VLS after administration of TBZ alone and coadministration of TBZ and deprenyl.

4.2 Materials and Methods

Animals

Adult male Sprague Dawley rats (N=26) weighing 350-450g during the course of the experiment had ad libitum access to lab chow and water (Harlan Laboratories, Indianapolis, IN). They were pair-housed in a colony that was maintained at approximately 23°C and had a 12-hour light/dark cycle (lights on at 0700 hrs). These studies were conducted according to University of Connecticut and NIH guidelines for animal care and use.

Drug Treatment Procedures and Dose Selection

Tetrabenazine was purchased from Tocris Bioscience (Bristol, UK) and dissolved in a vehicle solution of 0.9% saline (80%) and dimethylsulfoxide (DMSO) (20%). Ten µl hydrochloric acid (HCl)/mL volume was then added to get the drug completely into solution.

Deprenyl was purchased from Tocris Bioscience (Bristol, UK) and dissolved in 0.9% saline. The dose of tetrabenazine (2.0 mg/kg) used in these experiments was based on data from Podurgiel et al., 2013, showing this dose significantly induces TJMs. Doses of deprenyl were based on extensive pilot work.

Behavioral Procedure: Tremulous Jaw Movements

Observations of rats took place in a 30 × 30 × 30 cm clear Plexiglas chamber with a wire mesh floor, which was elevated 42 cm from the table top. This allowed for the viewing of the animal from several angles, including underneath. TJMs were defined as rapid vertical deflections of the lower jaw that resembled chewing but were not directed at any particular stimulus (Salamone *et al*, 1998). Each individual deflection of the jaw was recorded using a mechanical hand counter by a trained observer, who was blind to the experimental condition of the rat being observed. Separate studies with two observers demonstrated an inter-rater reliability of $r = 0.999$ ($p < 0.01$) using these methods.

Surgical Procedures

Before surgery, rats were anesthetized with a 1.0 ml/kg IP injection of a cocktail solution containing 10.0 ml of 100 mg/mL ketamine plus 0.75 ml of 20.0 mg/ml xylazine (both from Phoenix Scientific, Inc., St. Joseph, MO, USA). Rats were placed in a stereotaxic frame (Kopf, Tujunga, CA, USA; incisor bar 5.0 mm above interaural line), and received unilateral implantations of a 10.0 mm probe guide cannula (Bioanalytical Systems, Indianapolis, IN, USA). The tips of the guide cannulae were implanted into the ventrolateral striatum (AP: +1.4 mm, ML: ±3.8 mm, DV: −5.2 mm from bregma; counterbalanced for left vs. right) and were secured

to the skull with stainless steel screws and cement. Stainless steel stylets were inserted into the guide cannulae to maintain patency. Following surgery, animals were housed in separate cages and allowed 7 days post-surgical recovery.

DA Microdialysis and HPLC

Rats with implanted cannulae were placed in Plexiglas chambers (28 x 28 x 23 cm) the day before sampling for 8 hours of habituation with the infusion pumps running. The following day, the probe was inserted through the cannula, and artificial cerebrospinal fluid was pumped through at a rate of 2 μ L/min using a syringe pump (Harvard Apparatus, Cambridge, MA). Two hours post-insertion, sampling began and continued for 7 hours. Following the end of the sampling session, the probe was removed, and after euthanasia histological analyses were performed to verify placement sites. Samples were frozen and analyzed for DA content using reverse-phase HPLC with electrochemical detection (ESA, New Bedford, MA, USA; channel 1: -100 mV, channel 2: +200 mV, guard cell: +350 mV). Each liter of mobile phase contained 27.6g sodium phosphate monobasic monohydrate, 8% methanol, 750 μ L 0.1 M EDTA, and 2000 μ L 0.4 M sodium octyl sulfate (SOS) dissolved in dH₂O (pH=4.5). Flow rate was 1.0 mL/min. DA standards were assayed before, during, and after the collection of dialysis samples.

Experiments

Experiment 1: Ability of the MAO-B inhibitor deprenyl to attenuate tetrabenazine-induced tremulous jaw movements

A group of 8 rats was used to assess the ability of the MAO-B inhibitor deprenyl to attenuate tremulous jaw movements induced by the acute administration of TBZ (2.0 mg/kg). A

within-groups design was utilized for this study, with all rats receiving all drug treatments in a randomly varied order (one treatment per week; no treatment sequences repeated). On the test day, each rat received an IP injection of either 1.0 ml/kg vehicle solution (80% saline, 20% DMSO) or 2.0 mg/kg TBZ. Ninety minutes later, rats received an IP injection of either 1.0 ml/kg 0.9% saline (vehicle), 7.5, or 15 mg/kg deprenyl. Twenty minutes after the second injection, rats were placed in the Plexiglas observation chamber and allowed to habituate for 10 minutes. TJMs were then counted for 15 minutes, with the observation period divided into three 5-min epochs.

Experiment 2: Neurochemical analyses of extracellular DA in the VLS after administration of TBZ and deprenyl

Rats were operated with dialysis guide cannulae, allowed to recover, and had dialysis probes inserted on the morning of the drug test. On test days, rats (n=18) were randomly assigned to one of three treatment conditions: Veh/Veh (n=5), TBZ/Veh (n=6), or TBZ/Dep (n=7). After collection of sample 6, rats received an IP injection of either 1.0 ml/kg vehicle solution (80% saline, 20% DMSO) or 2.0 mg/kg TBZ. Ninety minutes later, rats received an IP injection of either 1.0 ml/kg 0.9% saline (vehicle), or 15 mg/kg deprenyl.

Data Analysis

The data for experiment 1 was analyzed using a repeated measures analysis of variance (ANOVA). Average TJMs per five-minute observation period were calculated and used in the ANOVA calculations. A computerized statistical program (SPSS 21.0 for Windows) was used to perform these analyses. When there was a significant ANOVA, planned comparisons using the overall error term were used to assess the differences between each dose and the control

condition (the number of comparisons was restricted to the number of treatments minus one (Keppel, 1991). For experiment 2, an ANOVA was used to test for differences in baseline DA levels between the three conditions. Changes in DA levels were calculated as percentage change from baseline. Three repeated measures factorial ANOVAs were performed: Veh/Veh vs TBZ/Veh; TBZ/Veh vs TBZ/Dep; Veh/Veh vs TBZ/Dep. When there was a significant interaction, analysis of simple effects was used to compare differences between the two conditions at each 30 minute sample.

4.3 Results

Experiment 1: Ability of the MAO-B inhibitor deprenyl to attenuate tetrabenazine-induced tremulous jaw movements

Repeated measures ANOVA revealed a significant overall effect of deprenyl treatment on TBZ-induced TJMs ($F(3,21) = 12.496$; $p < 0.0001$). 2.0 mg/kg TBZ significantly induced TJMs compared to vehicle control (planned comparisons, $p < 0.05$) and deprenyl (15.0 mg/kg) significantly reduced TBZ-induced TJMs (planned comparisons, $p < 0.05$) (Figure 4.1).

Experiment 2: The effect of TBZ and deprenyl administration on extracellular dopamine levels in the ventrolateral striatum

ANOVA revealed no differences in baseline DA levels (nanograms) in the ventrolateral striatum across conditions. ($F(2,17) = 1.064$, $p > 0.05$; Veh/Veh: 0.025 ± 0.007 ; TBZ/Veh: 0.026 ± 0.006 ; TBZ/Dep: 0.044 ± 0.013). Repeated measures factorial ANOVA revealed a significant effect of sample ($F(6, 90) = 10.654$; $p < 0.001$), a significant treatment by sample interaction ($F(12, 90) = 3.398$; $p < 0.001$), but no significant effect of treatment. To identify the source of the

interaction, individual repeated measures factorial ANOVAs comparing each of the treatment groups (Veh/Veh vs TBZ/Veh; TBZ/Veh vs TBZ/Dep; Veh/Veh vs Veh/Dep) were performed.

Repeated measures factorial ANOVA revealed a significant effect of treatment ($F(1, 9) = 171.0991$; $p < 0.001$) and sample ($F(6, 54) = 12.212$; $p < 0.001$) and a significant treatment by sample interaction ($F(6, 54) = 8.772$; $p < 0.001$) between animals in the Veh/Veh vs. TBZ/Veh conditions. Analysis of simple effects revealed that administration of TBZ increased extracellular DA in sample 1 ($F(1, 9) = 7.63$; $p < 0.05$), and decreased extracellular DA in the VLS compared to the vehicle condition in samples 3 ($F(1, 9) = 5.914$; $p < 0.05$), 4 ($F(1, 9) = 8.755$; $p < 0.05$), and 5 ($F(1, 9) = 7.273$; $p < 0.05$).

Repeated measures factorial ANOVA revealed a significant effect of treatment ($F(1, 11) = 111.149$; $p < 0.001$) and sample ($F(6, 66) = 12.428$; $p < 0.001$) and a significant treatment by sample interaction ($F(6, 66) = 2.433$; $p < 0.05$) between animals in the TBZ/Veh and TBZ/Dep conditions. Analysis of simple effects revealed that coadministration of TBZ plus deprenyl increased extracellular DA in the VLS compared to TBZ alone in sample 5 ($F(1, 11) = 5.400$; $p < 0.05$). Repeated measures factorial ANOVA revealed a significant effect of treatment ($F(1, 10) = 97.428$; $p < 0.001$) sample ($F(6, 60) = 2.556$; $p < 0.05$) but no treatment by sample interaction between animals in the Veh/Veh and TBZ/Dep conditions.

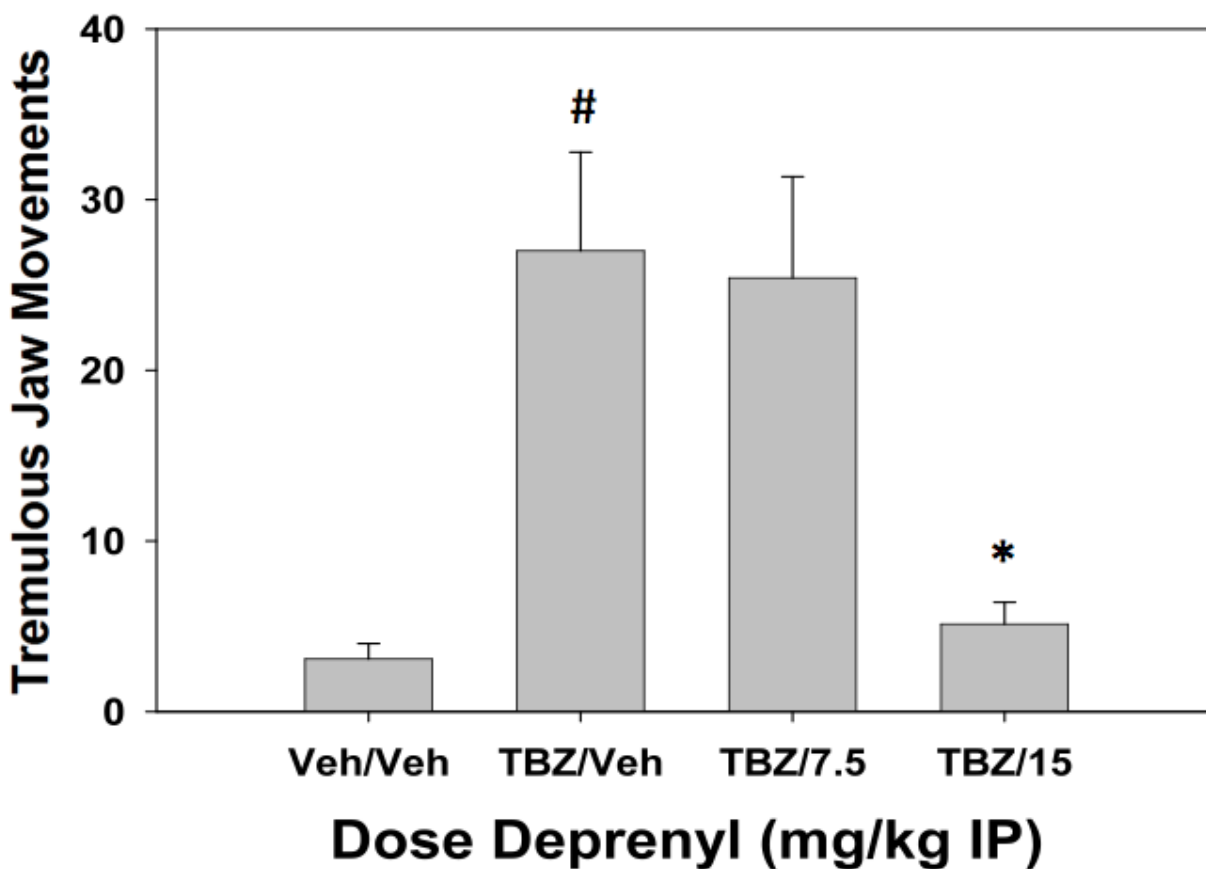


Figure 4.1: Effect of MAO-B inhibition on tetrabenazine (2.0 mg/kg) induced TJMs. Mean (\pm SEM) number of jaw movements (per 5 min) in rats treated with vehicle (Veh/Veh), tetrabenazine plus vehicle (TBZ/Veh), and tetrabenazine plus various doses (7.5, 15.0 mg/kg) of deprenyl. #significant difference from Veh/Veh ($p < .05$); *significant difference from TBZ/Veh ($p < 0.05$)

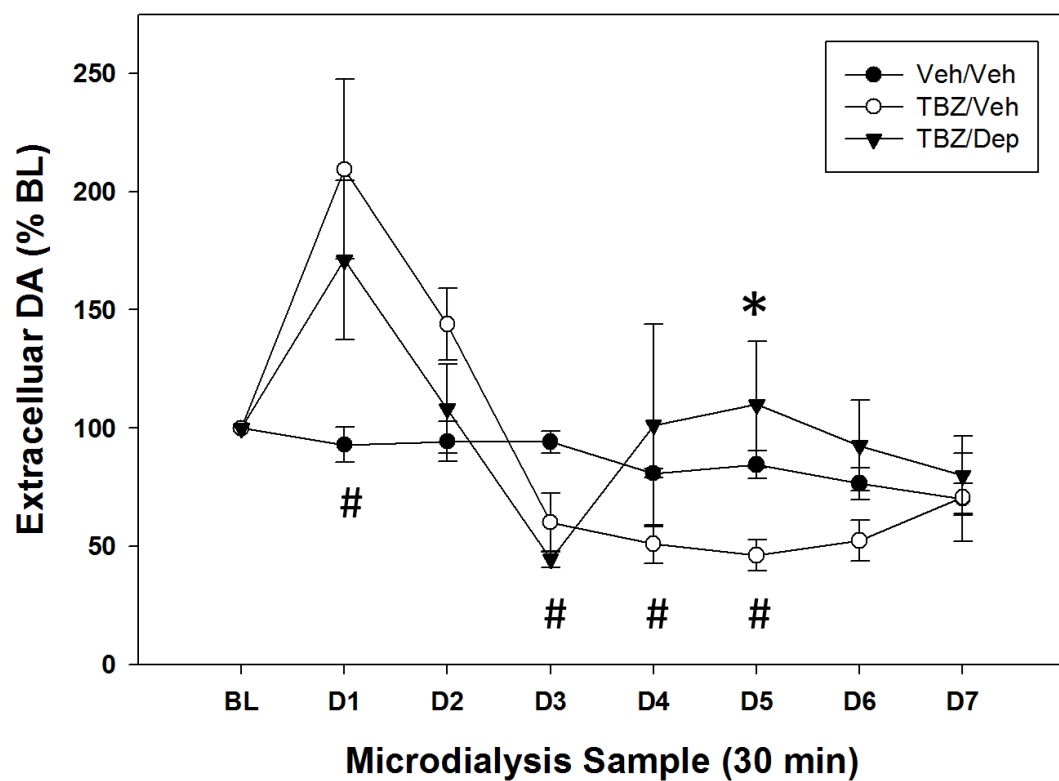


Figure 4.2: Effect of tetrabenazine and deprenyl on extracellular DA in VLS. Mean (\pm SEM) extracellular DA (expressed as percent baseline) in 30-minute samples. A baseline sample was collected prior to injection, followed by seven post-drug samples. #significant difference from Veh/Veh ($p < 0.05$); *significant difference from TBZ/Veh ($p < 0.05$)

Chapter 5: Fluoxetine administration exacerbates oral tremor and striatal dopamine depletion in a rodent pharmacological model of Parkinsonism

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5.1 Introduction

Idiopathic Parkinson's Disease (PD) is caused by a progressive degeneration of the dopamine (DA) producing neurons of the substantia nigra *pars compacta*. In addition to idiopathic PD, there also are drug-induced forms of Parkinsonism, which are caused by administration of drugs that block DA receptors or deplete DA. Parkinsonism is a family of motor disorders that is characterized by several cardinal motor symptoms, including resting tremor, akinesia, bradykinesia, and rigidity (Marsden et al., 1975; Ostrem and Galifianakis, 2010). These motor abnormalities can be modeled in rodents using various behavioral paradigms. Locomotor activity can be examined as an indicator of akinesia/bradykinesia, and the tremulous jaw movement (TJM) model can be used to evaluate resting tremor. TJMs are defined as rapid vertical deflections of the lower jaw that resemble chewing but are not directed at any particular stimulus (Salamone et al., 1998) and are generated by conditions that parallel those that induce Parkinsonism in humans, including neurotoxic or pharmacological dopamine (DA) depletion, DA antagonism, and cholinomimetic administration (Jicha and Salamone, 1991; Salamone et al., 2008; Betz et al., 2009; Collins et al., 2011; Podurgiel et al., 2013a). TJMs occur in the frequency range characteristic of Parkinsonian resting tremor (3-7.5 Hz; Salamone et al., 1998; Cousins et al., 1998; Collins et al., 2011; Podurgiel et al., 2013a) and can be attenuated by administration of antiparkinsonian agents, including L-DOPA (Cousins et al., 1997), DA agonists (Cousins et al., 1997; Salamone et al., 2005), muscarinic antagonists (Cousins et al., 1997; Betz et al., 2009) and adenosine A_{2A} antagonists (Correa et al., 2004; Simola et al., 2004;

Tronci et al., 2007; Salamone et al., 2008; Betz et al., 2009; Collins et al., 2011, 2012; Podurgiel et al., 2013a).

While Parkinsonism is primarily characterized by the generation of the cardinal motor symptoms, patients with PD also suffer from a variety of significant non-motor symptoms, including autonomic dysfunction, sensory abnormalities, gastrointestinal issues, sleep disorders, and neuropsychiatric disturbances (Ostrem and Galifianakis, 2010; Barone, 2011).

Neuropsychiatric symptoms are common even in the earliest stages of the disease, and can considerably affect the daily functioning and overall quality of life of PD patients (Aarsland et al., 2009; Barone, 2011). Depression, in particular, has been identified as the most significant predictor of health-related quality of life in PD (Schrag et al, 2000; Chen and Marsh, 2013), and systematic review and analysis suggest that 35-40% of patients with PD also experience clinically significant symptoms of depression (Slaughter et al., 2001; Aarsland et al., 2009).

Selective serotonin reuptake inhibitors (SSRIs) are prescribed more often than any other class of antidepressants for PD patients (Veazey et al., 2005; Aarsland et al., 2009; Chen and Marsh, 2013). Yet controlled clinical trials, meta-analyses, and systematic review collectively suggest that SSRIs are no more effective than placebo in treating depression in the context of PD (Skapinakis et al., 2010; Aarsland et al., 2009). Furthermore, SSRI administration has been associated with a number of motor side-effects, and may be implicated in increased motor disability in PD patients (Leo, 1996; Richard et al., 1997; Govoni et al., 2001; Veazey et al., 2005; Aarsland et al., 2009). There are presently more than 100 published reports of “extrapyramidal” symptoms (e.g. dystonia, akathisia, dyskinesia, and Parkinsonism, including tremor) associated with SSRI treatment; fluoxetine (Prozac; FLX) has been implicated in the majority of these reports (Madhusoodanan et al., 2010).

FLX primarily functions as an inhibitor of the serotonin (5-HT) transporter, preventing uptake of 5-HT and ultimately resulting in increased activation of a variety of 5-HT receptors, including the 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors found throughout the striatum (Nutt et al., 1999; Alex and Pehek, 2007; More et al., 2014). Interactions between 5-HT and DA neurotransmission are strongly implicated in the generation of motor dysfunctions associated with FLX treatment (Morelli et al., 2011), as DA release and metabolite production are inhibited by increased synaptic levels of 5-HT (Govoni et al., 2001; Morelli et al., 2011). Activation of 5-HT_{2C} receptors has been linked to decreased DA synthesis, neural activity and release in the nigrostriatal and mesolimbic DA pathways (Alex and Pehek, 2007; More et al., 2014). In rodent models, FLX administration has been shown to potentiate haloperidol-induced catalepsy and bradykinesia in a dose-dependent manner (Tatara et al., 2012; More et al., 2014).

Thus, FLX treatment may result in increased motor deficits in PD patients due to 5-HT-mediated exacerbation of DA depletion and basal ganglia dysfunction. The present study sought to characterize this interaction using a pharmacological rodent model of Parkinsonism. Tetrabenazine (TBZ) is a reversible and selective inhibitor of the type-2 vesicular monoamine transporter (VMAT-2) that is used to treat chorea associated with Huntington's disease. Huntington's disease patients taking TBZ can experience adverse events, including Parkinsonian motor symptoms and depression (Kenney et al., 2007; Frank, 2009). Recent studies show that high doses of TBZ in rodents (e.g., 2.0 mg/kg in rats, 5.0-10.0 mg/kg in CD1 mice) can induce TJMs and suppress locomotor activity (Podurgiel et al., 2013a). In the present studies, Experiment 1 examined the effect of acute administration of FLX (2.5, 5.0, or 10.0 mg/kg) on TJMs and locomotor suppression induced by a low dose of TBZ (0.75 mg/kg) in rats; this low dose was selected because it is used to study rat models of the motivational symptoms of

depression (Nunes et al., 2013; Randall et al., 2014). To test the hypothesis that 5-HT₂ family receptors are involved in the neural mechanisms underlying this behavior, experiment 2 assessed the ability of the 5-HT_{2A/2C} antagonist mianserin to attenuate TJMs induced by co-administration of TBZ (0.75 mg/kg) and FLX (5.0 mg/kg). Experiment 3 examined tissue levels of DA in the rat ventrolateral neostriatum (VLS), which is the homologue of the ventral putamen and the striatal subregion most closely associated with the production of TJMs (Jicha and Salamone, 1991; Salamone et al., 1998, 2008; Simola et al., 2004, Betz et al., 2009), after administration of TBZ (0.75 mg/kg), FLX (5.0 mg/kg), and mianserin (5.0 mg/kg).

5.2 Materials and Methods

Animals

Adult male Sprague Dawley rats (N=34) weighing 350-450g during the course of the experiment had ad libitum access to lab chow and water (Harlan Laboratories, Indianapolis, IN). They were pair-housed in a colony that was maintained at approximately 23°C and had a 12-hour light/dark cycle (lights on at 0700 hrs). These studies were conducted according to University of Connecticut and NIH guidelines for animal care and use.

Drug Treatment Procedures and Dose Selection

Tetrabenazine (9,10-dimethoxy-3-(2-methylpropyl)-1,3,4,6,7, 11b hexahydrobenzo[a]quinolizin-2-one; TBZ), the VMAT-2 inhibitor, was purchased from Tocris Bioscience (Bristol, UK). TBZ was dissolved in a vehicle solution of 0.9% saline (80%) and dimethylsulfoxide (DMSO) (20%). Ten µl hydrochloric acid (HCl)/mL volume was then added to get the drug completely into solution. Fluoxetine ((±)-N-Methyl-γ-[4-

(trifluoromethyl)phenoxy]benzenepropanamine hydrochloride; FLX) was purchased from Sigma-Aldrich Corporation (Saint Louis, MO, USA). FLX was dissolved in 0.9% saline. Mianserin hydrochloride (1,2,3,4,10,14b-Hexahydro-2-methyldibenzo[c,f]pyrazino[1,2-a]azepine hydrochloride) was purchased from Tocris Bioscience (Bristol, UK). Mianserin was dissolved in 0.3% tartaric acid. A dose of 0.75 mg/kg TBZ was selected based on previous studies with animal models of the motivational symptoms of depression (Nunes *et al*, 2013; Randall *et al*, 2014), and also on studies showing that this dose was lower than those that produce substantial TJMs (Podurgiel *et al.*, 2013), and was low enough to have a preferential effect on DA levels (Tanra *et al.* 1995). The doses of FLX used in experiment 1 (2.5, 5.0, and 10.0 mg/kg) were selected based on extensive pilot work involving animal models of the motivational symptoms of depression (Yohn *et al*, unpublished data). Doses of FLX for experiment 2 were based on results from experiment 1. Doses of mianserin (2.5 and 5.0 mg/kg) were selected based on previous work conducted in our laboratory (Carlson *et al*, 2003).

Behavioral Procedures

Tremulous Jaw Movements. Observations of rats took place in a 30 × 30 × 30 cm clear Plexiglas chamber with a wire mesh floor, which was elevated 42 cm from the table top. This allowed for the viewing of the animal from several angles, including underneath. TJMs were defined as rapid vertical deflections of the lower jaw that resembled chewing but were not directed at any particular stimulus (Salamone *et al.*, 1998). Each individual deflection of the jaw was recorded using a mechanical hand counter by a trained observer, who was blind to the experimental condition of the rat being observed. Separate studies with two observers demonstrated an inter-rater reliability of $r = 0.999$ ($p < 0.01$) using these methods.

Locomotor Activity. Locomotor activity was assessed by placing the rat in an automated activity chamber (28 × 28 × 28 cm) enclosed in a sound-attenuating shell. The floor of the chamber was elevated 6 cm above the chamber bottom and was composed of two moveable wire-mesh panels (25 × 12 cm) that were further divided into four quadrants by means of a central metal rod. As the rat entered each quadrant, a slight vertical movement of the mesh panels closed a microswitch located outside of the locomotion chamber. Each switch closure was detected and recorded by a computer program, written in MedPC (Med Associates, Inc., Georgia, VT, USA), as a single activity count. The locomotor activity session was 18-min in length. These methods of measuring locomotion have been used previously to assess the effects of DA antagonists and TBZ on locomotion (Salamone et al., 2008; Podurgiel et al., 2013a).

Tissue Collection and HPLC

Rats were exposed to carbon dioxide for 30 s and decapitated. Brains were quickly removed and frozen on a Leitz Wetzlar microtome. Coronal sections 750 µm thick were cut through the ventrolateral neostriatum (VLS). A 16-gauge stainless steel tube was used to dissect bilateral cylindrical samples from the VLS. The VLS was selected because of a substantial literature showing that this site is the most critical striatal subregion involved in the regulation of TJMs. Several papers have included placement controls, including injections into multiple striatal sites, multiple drugs or lesion methods, and injections into control sites dorsal to the VLS (Kelley et al., 1989; Salamone et al., 1990; Jicha and Salamone 1991; Cousins et al., 1998; Simola et al., 2004). Since previous work has shown that DA depletions could induce TJMs when 6-OHDA was injected into the VLS, but not other striatal sites (Jicha and Salamone, 1991), it was decided that the VLS was the critical neostriatal locus upon which to focus for studies

involving DA tissue levels. These tissue samples were then placed in 200 μ L of 0.1 N perchloric acid, and then homogenized, centrifuged and frozen. The supernatant was subsequently analyzed using high-performance liquid chromatography with electrochemical detection (HPLC-EC; ESA Coulochem II system). The electrochemical parameters were as follows: channel 1 = -100 mV, channel 2 = +200mV, guard cell = +350 mV. Each liter of mobile phase contained 27.6 g sodium phosphate monobasic, 8.0% methanol, 750 μ L of 0.1 M EDTA, and 2875 μ L of 0.4 M sodium octyl sulfate dissolved in deionized ultrapure H₂O with a final pH of 4.5. The flow rate was 1.0 ml/min.

Experiments

Experiment 1: Ability of FLX to exacerbate TBZ-induced TJMs tremulous jaw movements and locomotor suppression

A group of 12 rats was used to assess the effects of the acute administration of FLX on the motor symptoms induced by 0.75 mg/kg TBZ. A within-groups design was utilized for this study, with all rats receiving all drug treatments in a randomly varied order (one treatment per 3 week block; no treatment sequences repeated). On the test day, which occurred once every 3 weeks, each rat received an IP injection of either 1.0 ml/kg vehicle solution (80% saline, 20% DMSO) or 0.75 mg/kg TBZ. Thirty min later, rats received an IP injection of either 1.0 ml/kg 0.9% saline (vehicle) or 2.5, 5.0, or 10.0 mg/kg FLX. Thus, there were six conditions being studied (vehicle/vehicle, TBZ/vehicle, vehicle/10.0 mg/kg FLX, and TBZ with either 2.5, 5.0 or 10.0 mg/kg FLX). One hour and twenty min after the second injection, rats were placed in the Plexiglas observation chamber and allowed to habituate for 10 min. TJMs were then counted for 15 minutes, with the observation period divided into three 5-min epochs. Upon completion of the

TJM assessment, locomotor activity was assessed in the same group of 12 rats in an 18-min session using the procedure outlined above.

Experiment 2: Ability of mianserin to attenuate TJMs induced by co-administration of TBZ and FLX

A group of 8 rats was used to assess the ability of the 5-HT_{2A/2C} antagonist mianserin to attenuate TJMs induced by the acute co-administration of TBZ (0.75 mg/kg) and FLX (5.0 mg/kg). A within-groups design was used, with all rats receiving all drug treatments in a randomly varied order (one treatment per 3 week block; no treatment sequences repeated). On the test day, which occurred once every 3 weeks, each rat received an IP injection of either 1.0 ml/kg vehicle solution (80% saline, 20% DMSO) or 0.75 mg/kg TBZ. Thirty minutes later, rats received an IP injection of either 1.0 ml/kg 0.9% saline (vehicle) or 2.5, 5.0, or 10.0 mg/kg FLX. Fifty min later, rats received a subcutaneous injection of either 1.0 ml/kg 0.3% tartaric acid (vehicle) or 2.5 or 5.0 mg/kg mianserin. Thirty min after the third injection, rats were placed in the Plexiglas observation chamber and allowed to habituate for 10 min. TJMs were then counted for 15 min, with the observation period divided into three 5-min epochs.

Experiment 3: Neurochemical analyses for tissue DA after administration of TBZ, FLX, and mianserin

A total of 34 rats were used to examine tissue levels of DA after administration of TBZ, FLX, and mianserin (the 12 rats from experiment 1, the 8 rats from experiment 2, and 14 additional rats that received drug treatments matching those of the animals used for the first two experiments; these additional animals were needed because the tissue assay experiments required

a larger total N due to the between-groups design). A between-groups design was utilized for this study, with rats being randomly assigned to one of four treatment conditions: Veh/Veh/Veh (n=9), TBZ/Veh/Veh (n=9), TBZ/FLX/Veh (n=8), or TBZ/FLX/Mianserin (n=8). Rats received an IP injection of either 1.0 ml/kg vehicle solution (80% saline, 20% DMSO) or 0.75 mg/kg TBZ. Thirty min later, rats received an IP injection of either 1.0 ml/kg 0.9% saline (vehicle) or 5.0 mg/kg FLX. Fifty min later, rats received a subcutaneous injection of either 1.0 ml/kg 0.3% tartaric acid (vehicle) or 5.0 mg/kg mianserin. Forty min later, tissue collection was performed. One week later, samples were analyzed for DA content using HPLC-EC as described above.

Data Analysis

The data for experiments 1 and 2 were analyzed using a repeated measures analysis of variance (ANOVA). Average TJMs per five-minute observation period were calculated and used in the ANOVA calculations. A computerized statistical program (SPSS 21.0 for Windows) was used to perform these analyses. When there was a significant ANOVA, planned comparisons using the overall error term were used to assess the differences between each dose and the control condition (Keppel, 1991; the number of comparisons was restricted to the number of treatments minus one (Keppel, 1991). For experiment 3, DA levels were expressed as nanogram/mg wet weight of tissue. Data was analyzed using a between groups analysis of variance (ANOVA). When there was a significant ANOVA, planned comparisons using the error term from the paired conditions were used to assess the differences between conditions.

5.3 Results

Experiment 1: Ability of FLX to exacerbate TBZ-induced TJMs and locomotor suppression

Repeated measures ANOVA revealed a significant overall treatment effect on TJMs (Figure 5.1; $F(5,55) = 19.307$; $p < 0.05$). The 0.75 mg/kg dose of TBZ significantly induced TJMs compared to vehicle controls (planned comparisons, $p < 0.05$). Co-administration of TBZ (0.75 mg/kg) with 2.5, 5.0, or 10.0 mg/kg doses of FLX significantly increased TJMs from TBZ alone (planned comparisons, $p < 0.05$). Repeated measures ANOVA also revealed a significant overall treatment effect for locomotion (Figure 5.2; $F(5,55) = 6.757$; $p < 0.05$). The 10.0 mg/kg dose of FLX significantly suppressed locomotor activity as compared to vehicle controls (planned comparisons, $p < 0.05$). Co-administration of 0.75 mg/kg TBZ and 2.5, 5.0, or 10.0 mg/kg doses of FLX significantly reduced locomotor activity compared to TBZ alone (planned comparisons, $p < 0.05$).

Experiment 2: Ability of mianserin to attenuate TJMs induced by coadministration of TBZ and FLX

Repeated measures ANOVA revealed a significant overall treatment effect on TJMs (Figure 5.3; $F(4,28) = 3.451$; $p < 0.05$). Co-administration of 0.75 mg/kg TBZ and 5.0 mg/kg FLX significantly increased TJMs from the vehicle condition (planned comparisons, $p < 0.05$) and TBZ alone (planned comparisons, $p < 0.05$). Both doses of mianserin (2.5, 5.0 mg/kg) significantly reduced TJMs induced by co-administration of TBZ (0.75 mg/kg) and FLX (5.0 mg/kg) (planned comparisons, $p < 0.05$).

Experiment 3: Neurochemical analyses for tissue DA levels after administration of TBZ, FLX, and mianserin

Between subjects ANOVA revealed a significant overall treatment effect on DA levels in the VLS (Figure 5.4; $F(3,30) = 21.489$; $p < 0.0001$). Administration of TBZ (0.75 mg/kg) decreased DA levels in the VLS compared to the vehicle condition ($F(1,16) = 4.49$; $p < 0.05$). Co-administration of TBZ (0.75 mg/kg) and FLX (5.0 mg/kg) further decreased DA levels in the VLS compared to TBZ alone ($F(1,15) = 4.54$; $p < 0.05$). Co-administration of mianserin (5.0 mg/kg) with TBZ (0.75 mg/kg) and FLX (5.0 mg/kg) increased DA levels compared to TBZ and FLX alone ($F(1,14) = 4.60$; $p < 0.05$).

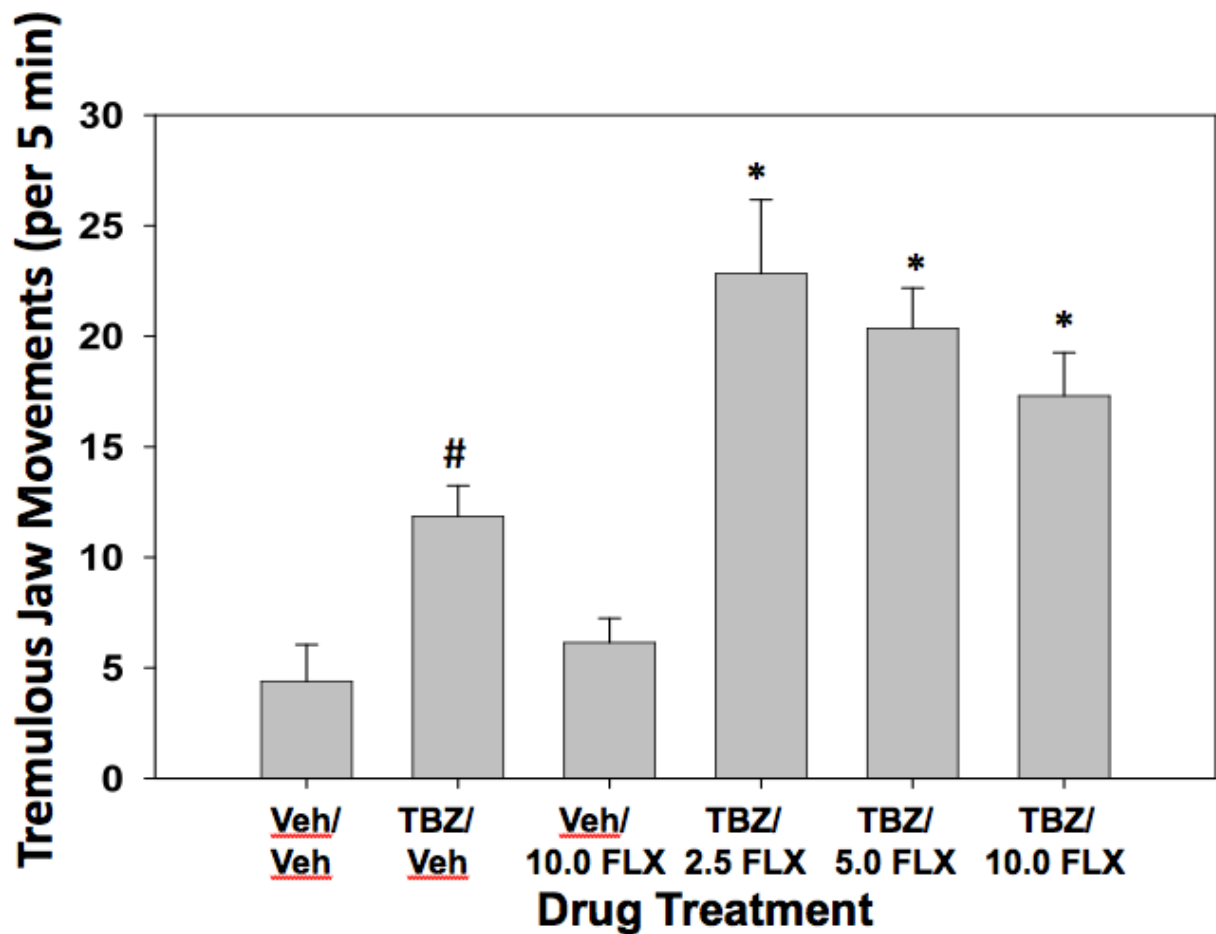


Figure 5.1: Mean (+ SEM) number of TJMs per 5 min observation period in rats that received injections of vehicle (Veh/Veh), 0.75 mg/kg tetrabenazine (TBZ/Veh), or various mg/kg doses of fluoxetine (FLX; 2.5-10.0 mg/kg) in combination with TBZ.

different from Veh/Veh, $p < 0.05$; * TBZ/FLX different from TBZ/Veh, $p < 0.05$

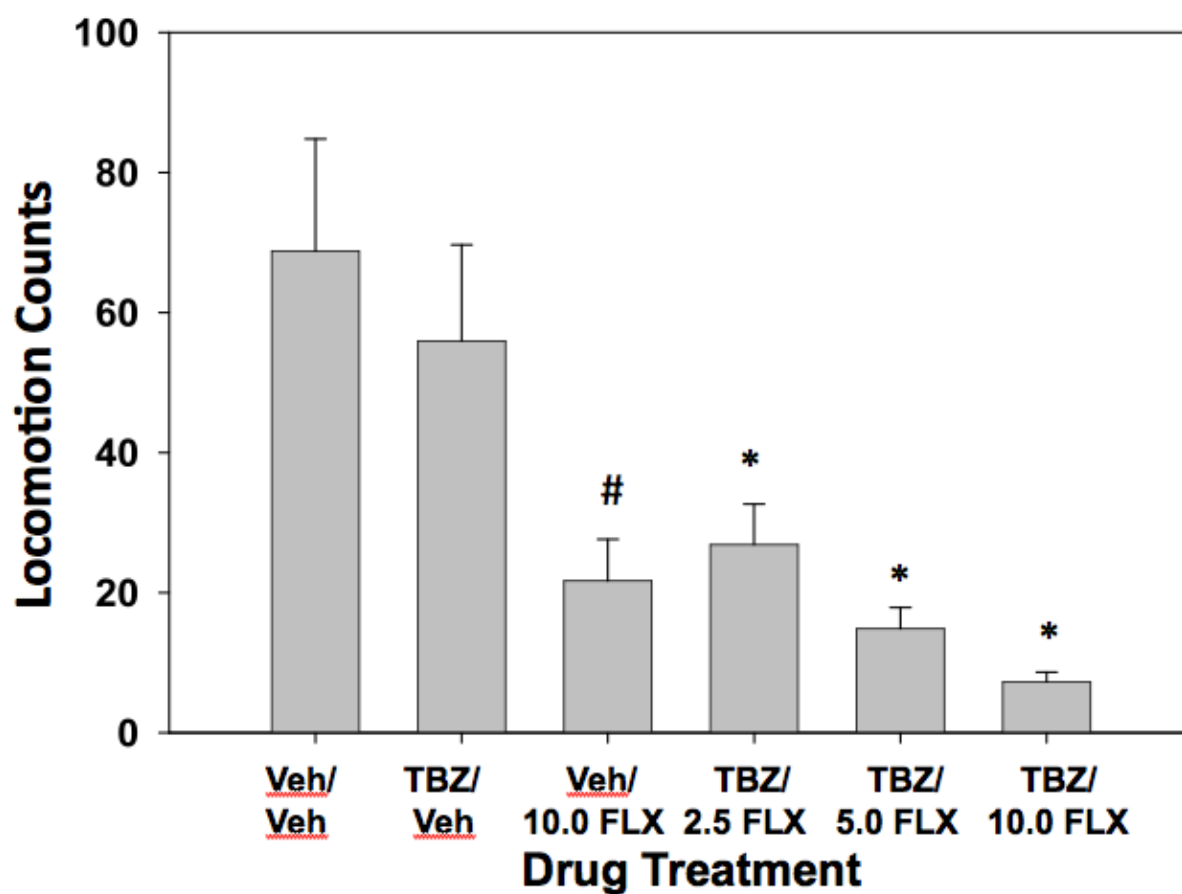


Figure 5.2: Mean (+ SEM) number of locomotor activity counts in rats that received injections of vehicle (Veh/Veh), 0.75 mg/kg tetrabenazine (TBZ/Veh), or various mg/kg doses of fluoxetine (FLX; 2.5-10.0 mg/kg) in combination with TBZ.

different from Veh/Veh, $p < 0.05$; * TBZ/FLX different from TBZ/Veh, $p < 0.05$

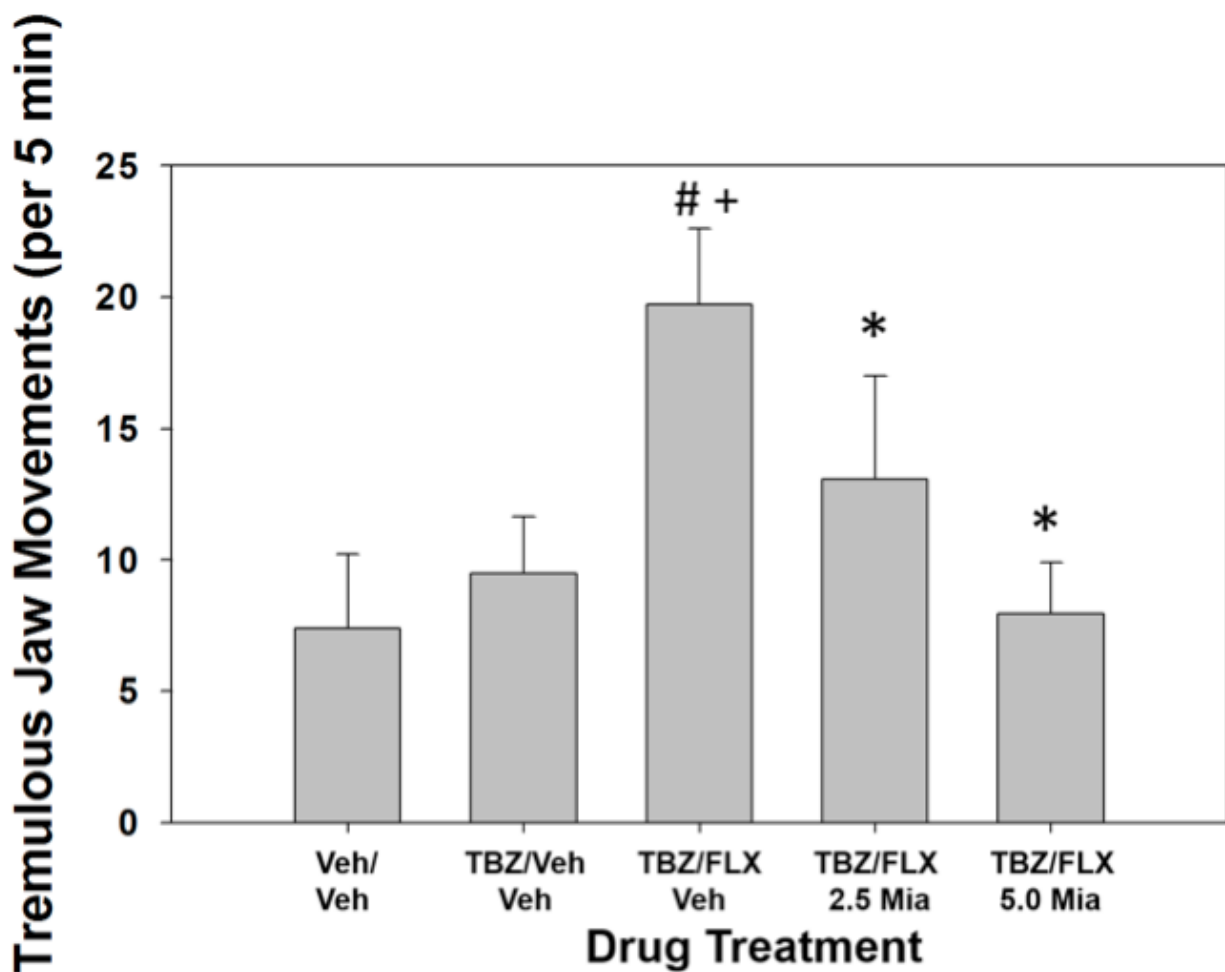


Figure 5.3: Mean (+ SEM) number of TJMs per 5 min observation period in rats that received injections of vehicle (Veh/Veh), 0.75 mg/kg tetrabenazine (TBZ/Veh), 5.0 mg/kg fluoxetine (FLX), and various doses of mianserin (Mia; 2.5-5.0 mg/kg) in combination with TBZ and FLX. # different from Veh/Veh, $p < 0.05$; + TBZ/FLX/Veh different from TBZ/Veh/Veh, $p < 0.05$; * TBZ/FLX/Mia different from TBZ/FLX/Veh, $p < 0.05$

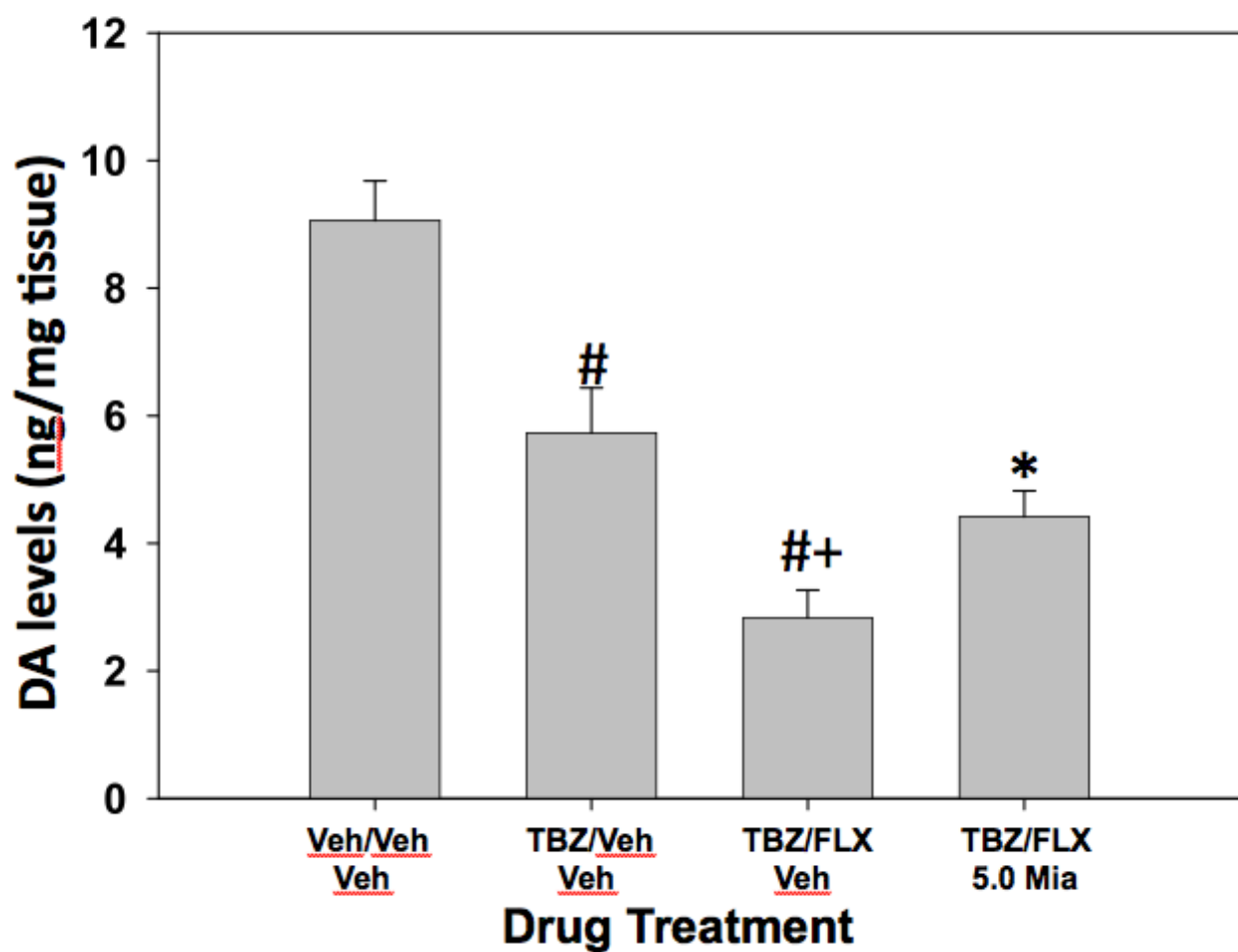


Figure 5.4: Mean (+ SEM) amount of DA in ventrolateral neostriatum (ng/mg tissue) in rats that received injections of vehicle (Veh/Veh), 0.75 mg/kg tetrabenazine (TBZ/Veh), 5.0 mg/kg fluoxetine (FLX), and 5.0 mg/kg mianserin (Mia) in combination with TBZ and FLX.

different from Veh/Veh, $p < 0.05$; + TBZ/FLX/Veh different from TBZ/Veh/Veh, $p < 0.05$;

* TBZ/FLX/Mia different from TBZ/FLX/Veh, $p < 0.05$

Chapter 6: Tremor-related subthalamic and cortical local field potentials associated with pilocarpine-induced oral tremor

6.1 Introduction

Parkinsonism is a broad family of disorders that includes idiopathic Parkinson's disease (PD), which results from degeneration of nigrostriatal dopamine (DA) neurons (Hornykiewicz, 1973), as well as drug-induced Parkinsonism (DIP). DIP is induced by drugs that interfere with DA transmission (e.g. DA antagonists, DA depleting agents; Marsden et al., 1975; McEvoy, 1983), and cholinomimetics such as anticholinesterases and muscarinic agonists (Ott and Lannon, 1992; Aarsland et al., 2003). The cardinal motor symptoms of Parkinsonism include akinesia, bradykinesia, rigidity, and resting tremor, which typically occurs in the 3-7 Hz frequency range (Marsden et al., 1975). DIP is produced in rodents by the same pharmacological agents that induce human Parkinsonism, and resting tremor can be modeled in rodents using the tremulous jaw movement (TJM) model. TJMs, defined as "rapid, repetitive vertical deflections of the lower jaw that resemble chewing but are not directed at any particular stimulus" (Salamone et al., 1998), have a local frequency of 3-7 Hz, which parallels that of Parkinsonian resting tremor (Salamone et al., 1998; Cousins et al., 1998; Collins et al., 2010; Podurgiel et al., 2013a). TJMs are induced by neurotoxic or pharmacological depletion of DA, DA antagonism, and cholinomimetic drugs (Jicha and Salamone 1991; Salamone et al., 1998; Podurgiel et al., 2013b, 2015), and can be attenuated by co-administration of antiparkinsonian agents such as L-DOPA, DA agonists, muscarinic antagonists, MAO inhibitors, and adenosine A_{2A} antagonists (Cousins et al., 1997; Simola et al., 2004, 2006; Salamone et al., 2005, 2008a,b; Podurgiel et al., 2013a,b). Considerable evidence indicates that TJMs in rodents are a valid model for the

exploration of the pharmacology, neurochemistry and physiology of drug-induced tremor (Salamone et al., 1998, 2008b, 2013; Collins-Praino et al., 2011; Podurgiel et al., 2013a,b, 2015).

Exaggerated neuronal synchrony has been recorded in basal ganglia and cortex of PD patients, with the beta band (~15-30 Hz) being the best-characterized oscillation (Brown 2003; Hammond et al., 2007; Oswal et al., 2013). Increased beta activity has been observed in the cortex (George et al., 2013) and subthalamic nucleus (STN) of PD patients, and it has been suggested that excessive synchrony in this frequency range contributes to motor dysfunction (Levy et al., 2002; Brown and Williams, 2005; Kuhn et al., 2006). Reduction in STN beta activity correlates with improvement in akinesia and rigidity in PD patients (Levy et al., 2002; Kuhn et al., 2006). While the literature supports a link between increased cortical and basal ganglia beta power and the development of akinesia/rigidity, beta activity generally does not correlate with the severity of resting tremor (Kuhn et al., 2005; Hammond et al., 2007; Oswal et al., 2013). Rather, the development of tremor in PD patients has been shown to be associated with the emergence of oscillations in the tremor frequency range (3-7 Hz) in the cortex and basal ganglia (Timmerman et al., 2003; Reck et al., 2009; Hirschmann et al., 2013; Oswal et al., 2013). Timmerman et al. (2003) reported strong coherence between electromyograph (EMG) activity of forearm muscles and activity in the contralateral primary motor cortex (M1), at tremor (3-7 Hz) and double tremor frequency (7-13 Hz) in PD patients off medication. Similar patterns of activity have been observed in the STN of PD patients, as indicated by power spectra peaks at tremor frequency and tremor harmonics, as well as significant coherence between STN local field potentials (LFPs) and EMG activity at tremor frequency (Brown et al., 2001; Levy et al., 2000; Liu et al., 2002; Wang et al., 2005; Reck et al., 2009).

These clinical reports support the idea that cortical and STN power and coherence at tremor frequencies increase with the manifestation of tremor, but this phenomenon has not been modeled in rodents. Therefore, the present study characterized the temporal pattern of oral EMG activity and associated changes in LFPs recorded from M1 and STN during the TJMs induced by the muscarinic agonist pilocarpine (Collins et al., 2010; Collins-Praino et al., 2012; Salamone et al., 2013).

6.2 Materials and Methods

Animals

A total of 5 adult male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) with no prior drug experience were used in the present experiment. The rats weighed 350-450 g during the course of the experiment and had *ad libitum* access to lab chow and water. Animals were group-housed prior to surgery in a colony that was maintained at approximately 23°C and had a 12-hour light/dark cycle (lights on at 0700 hrs). Post-surgery, animals were single housed to avoid over grooming around the surgical implant. This study was conducted according to University of Connecticut and NIH guidelines for animal care and use.

Drug Treatment Procedures and Dose Selection

Pilocarpine was purchased from Sigma Aldrich Chemical (St. Louis, MO) and dissolved in 0.9% saline. The dose of pilocarpine (4.0 mg/kg) was based on previous experiments showing significant induction of jaw movements at this dose (see Collins et al. 2010a for further details).

Surgical Procedures

Rats were anesthetized with a 1.0 ml/kg IP injection of a cocktail solution containing 10.0 ml of 100 mg/mL ketamine plus 0.75 ml of 20.0 mg/ml xylazine (Phoenix Scientific, Inc., St. Joseph, MO, USA). Rats were placed in a stereotaxic frame (Kopf, Tujunga, CA, USA), and a midline scalp incision was made. Two electrode arrays consisting of 50 μ m tungsten wire (California Fine Wire Company, Grover Beach, CA) were bilaterally implanted with a 27-gauge needle approximately 5.0 mm deep into the lateral temporalis muscle (4 EMG electrodes per animal). Previous research has demonstrated that the lateral temporalis muscle is the jaw muscle that shows activity most closely related to TJMs (Cousins et al., 1998). Burr holes were drilled through the skull over the STN (R hemisphere) and M1 (L hemisphere), and two –four electrode arrays were implanted (8 LFP electrodes per animal). LFP electrode arrays were comprised of four linearly spaced 50 μ m tungsten wires (California Fine Wire Company, Grover Beach, CA). Electrode wire was arranged and separated by fused silica tubing (Polymicro Tubing, Phoenix, AZ), attached to female pins (Omnetics, Minneapolis, MN) and secured in a rectangular five by four pin array. Two stainless steel watch screws driven into the skull above the cerebellum served as indifferent and ground electrodes. Supplementary anchor screws were positioned as necessary and the entire head-stage ensemble was fortified with dental acrylic. The surgical coordinates, for which bregma and the top of the skull was used as the reference point, were as follows: STN (AP: -3.6, ML: +/- 2.5, DV: -7.5); M1 (AP +1.0, ML +1.9, DV -2.5). Rats recovered for one week post-surgical procedure.

Behavioral Measures

Following a one-week recovery period, rats were given an acute IP injection of saline (vehicle). Immediately after vehicle injection, rats were placed into a Plexiglas observation chamber and allowed to habituate for 10 min. At the beginning of this habituation period, the animals were connected to the recording apparatus by a multi-channel tether (Neuralynx, Bozeman, MT) that was attached to a pulley system in the ceiling. Following the habituation period, a trained observer counted tremulous jaw movements for fifteen minutes. TJMs were defined as rapid vertical deflections of the lower jaw that resembled chewing but were not directed at any particular stimulus (Salamone et al. 1998). At the end of the 15-minute observation period, rats were disconnected and returned to their home cages. This procedure was repeated with administration of 4.0 mg/kg pilocarpine 24 hours later.

Electrophysiological Data Acquisition and Analysis

Following the habituation period, wide-band electrical activity was recorded (5050.5 samples/sec) for 15 minutes using a Neuralynx data acquisition system (Bozeman, MT). TJMs were counted simultaneously by a trained observer and noted using event markers through Cheetah data acquisition software (version 5.6.3; Neuralynx, Bozeman, MT). Following data acquisition and during subsequent offline analysis, data were imported into Matlab R2014a (Mathworks, Natick, MA). The raw EMG signal was bandpass filtered between 500 and 1500 Hz and the Hilbert transform was computed on the bandpass filtered signal. In this regard, the instantaneous (5050.5 samples/sec) EMG envelope amplitude (magnitude of Hilbert transform) was obtained over time. The EMG signal was then full wave rectified and all raw EMG traces presented in the current analysis represent this full wave rectified signal. Event markers were

simultaneously imported into Matlab and plotted along with the EMG signal. The presence or absence of these event markers was used to identify TJM and no TJM epochs, respectively. Raw LFP data were imported into Matlab R2014a and down-sampled by a factor of 10 during offline analysis, thus changing the sampling rate to 505.05 samples/s (Hz; $5050.5/10 = 505.05$). The raw LFP signal was lowpass filtered to remove high frequency chewing, chattering and/or teeth grinding artifacts ($F_c = 200$ Hz). Then, the LFP signal was bandpass filtered for tremor (3-7 Hz) and beta frequency (15-30 Hz) and the Hilbert transform was computed on the bandpass filtered signals. Data were examined during: 1) TJM epochs and, 2) No TJM epochs.

All data analysis was conducted using custom written programs in MatLab R2014a (Mathworks, Natick, MA). Power spectral density estimates were obtained using Welch's averaged modified periodogram method (Welch, 1967) during epochs of TJMs and no TJMs. To extract the amplitude modulation (AM) frequency of the EMG signal (e.g., "tremor frequency"; 3-7 Hz), power spectral density estimates were obtained from the envelope (magnitude of Hilbert transform) of the bandpass filtered EMG signal. For LFP data, the power for tremor (3-7 Hz) and beta frequency (15-30 Hz) was calculated from the bandpass filtered signals and represented in units of mV^2 . Average power was calculated by taking the sum of the power values within a given frequency range of interest (e.g, 3-7 Hz) and multiplying the sum by the spectral window resolution. Centroid frequency or the weighted mean was calculated by first isolating the frequency range of interest (e.g., 3-7 Hz), indexing the power values within that frequency range and multiplying them. Then, we divided those values by the sum of the previously indexed power values. Lastly, we took the sum of the aforementioned.

Statistics

For each of the 5 animals a representative 2.2 second TJM was identified by a trained observer using event markers. The TJM length was determined by the lowest responding animal. Further, within the same recording for each animal a subsequent 2.2 second no TJM time point was isolated as indicated by lack of event markers. For each electrode, the average power and centroid frequency was computed within the frequency range of interest (3-7 Hz and 15-30 Hz) for TJM and no TJM epochs all the while discretized by electrode location (M1 and STN). Paired samples t-tests were computed to assess if there were significant differences in 1) average tremor power during TJMs and no TJMs for M1, 2) average tremor power during TJMs and no TJMs for STN, 3) average beta power during TJMs and no TJMs for M1, 4) average beta power during TJMs and no TJMs for STN, and 5) the same as 1-4, but for centroid frequency. Further, we computed paired-samples t-tests to examine if there were differences in the aforementioned indices as a function of brain area (M1 and STN). Lastly, paired samples t-tests were used to confirm significant differences in number of TJMs per 15 minute recording period across vehicle and pilocarpine recordings.

Histology

At the completion of the experiment, animals were deeply anesthetized with CO₂ and perfused with 0.9% physiological saline followed by 3.7% formaldehyde solution. The brains were extracted and stored in the formaldehyde solution for 1 week. Then, brains were sliced (50 μ m sections) using a vibratome (Leica, Germany), mounted, Nissl stained using Cresyl Violet and cover-slipped allowing for verification of electrode placements. Photomicrographs of electrode tracks were taken using a Nikon microscope connected to a Spot RT camera system,

digitized and prepared for presentation using Adobe Photoshop. Consistent with the histological criteria employed by Brown et al. (2011) only placements that were within 500 μm of the STN, but dorsal to the cerebral peduncles and internal capsule were used for statistical analyses.

6.3 Results

Histological verification of electrode placements

A total of 37 LFP electrodes ($n=19$ M1 electrodes; $n=19$ STN electrodes) and 5 EMG electrodes across 5 animals were used in the current analysis. All animals contributed 1 EMG electrode, 3-4 M1 (Figure 6.1A) and 3-4 STN (Figure 6.1B) electrodes. All EMG and LFP data were simultaneously recorded from each animal. M1 electrodes terminated in all cortical layers but were more likely to terminate in deeper layers. Further, most STN electrodes were hits, although a few terminated slightly dorsally or anteriorly (data not shown), but still within the 500 μm criteria put forth by Brown et al. (2011).

Pilocarpine induces TJMs in the tremor frequency range (3-7 Hz) as reflected by EMG

Administration of pilocarpine significantly induced TJMs compared to vehicle control (Pilo mean: 603.6 ± 314.4 ; Veh mean: 14.4 ± 9.8 ; $t(4) = 4.3$, $p < .05$). Although vehicle conditions were used to verify the ability of pilocarpine to produce TJMs, large behavioral differences existed between vehicle and pilocarpine recordings that made electrophysiological comparisons between these two signals unsuitable (i.e., no tremor bursts, but more movement artifact, under vehicle conditions). Because of these differences, all EMG and LFP data analysis were conducted on epochs of TJMs and no TJMs within the pilocarpine recording for each animal.

During bouts of jaw movement activity, the bandpass filtered (500-1500 Hz) and full wave rectified EMG signal was marked by rhythmic activity in the 3-7 Hz range (Figure 6.2A top, left; red). Conversely, during periods of quiescence, no rhythmic activity was observed in the EMG (Figure 6.2A bottom, left; blue). Spectrograms indicated the same pattern of activity as the filtered EMG traces (Figure 6.2A, right). For the traces presented in Figure 6.2A, power spectral analysis revealed strong rhythmicity in the envelope of the EMG signal with a fundamental frequency of 4 Hz along with robust 2nd and 3rd harmonics (Figure 6.2B, red). Epochs of quiescence failed to exhibit amplitude modulation of the EMG signal within the tremor frequency range (Figure 6.2B, blue) and, show relatively little power overall. Upon examination of the entire pilocarpine recording for the same representative animal, rodents continued to exhibit dominant power of the EMG envelope within the tremor frequency range, although the harmonics were largely attenuated (Figure 6.2C). Importantly, this animal exhibited TJMs for 23.15% (3.47/15 minutes) of the recording session (Figure 6.2C right) indicating the robustness of the observed phenomenon.

Power in the tremor frequency band increases during TJMs in M1 and STN

Upon examination of simultaneously recorded M1 LFPs for the same animal as in Figure 6.2, the raw (Figure 6.3A top) and bandpass filtered (3-7 Hz; Figure 6.3A middle) LFP signals revealed increased power in the tremor frequency band during epochs of TJMs (Figure 6.3A left, red), but not during bouts of quiescence (Figure 6.3A right, blue). The LFP signal was indexed during epochs of TJMs and no TJMs as indicated by EMG event markers or lack thereof, respectively. Spectrograms of the raw signal revealed differential patterns of LFP activity for bouts of TJMs and quiescence (Figure 6.3A bottom). The power spectrum of the raw LFP signal

filtered for tremor frequencies (3-7 Hz) during epochs of TJMs (red) and no TJMs (blue) indicated strong LFP power in the tremor range with a peak at ~4 Hz for bouts of TJMs, while little LFP power existed at such frequencies for bouts lacking TJMs. At a simultaneously recorded STN site, the same pattern of activity was present (Figure 6.3B). Overall, the bandpass filtered LFP signal (3-7 Hz) during bouts of TJMs and the subsequent power spectrum of that signal revealed strong power in the 3-7 Hz range, whereas bouts of quiescence did not exhibit this effect (Figure 6.3B).

Summary data from all animals revealed the same pattern of effects. Overall, M1 LFPs during TJM epochs (red) exhibited significantly more power in the 3-7 Hz range as compared to no TJM (blue) epochs (Figure 6.3C; $t(18) = 5.13, p < .05$). The same pattern of activity existed for STN recording sites (Figure 6.3C, right; $t(18) = 4.55, p < .05$). Further, M1 exhibited more power in the tremor band during TJM and no TJM epochs compared to STN (TJM: $t(18) = 3.47, p < .05$; no TJM: $t(18) = 3.26, p < .05$). Analysis of centroid frequency (Figure 6.3D) revealed no differences between bouts of TJMs and no TJMs within a given brain area (e.g., M1 TJM vs. M1 no TJM; M1: $t(18) = -0.79, p > .05$; STN: $t(18) = -0.36, p > .05$). Moreover, there were no significant differences in LFP centroid frequency between TJM and no TJM epochs across brain areas (e.g., M1 TJM vs. STN TJM; TJM: $t(18) = 0.51, p > .05$; No TJM: $t(18) = 1.88, p > .05$).

Beta band power does not increase during TJMs in M1 and STN

Simultaneously recorded M1 LFPs for the animal shown in Figure 6.2 and 6.3, revealed similar LFP beta band power during epochs of TJMs (Figure 6.4A left, red) and epochs of quiescence (Figure 6.4A right, blue) for the raw (Figure 6.4A top) and bandpass filtered (15-30 Hz; Figure 6.4A middle) LFP signals. Importantly, the data shown here are for the same time

points as presented in Figure 6.2 and 6.3, but here data were filtered for beta (15-30 Hz) instead of tremor frequencies (3-7 Hz). A closer look at the signal revealed similar instantaneous fluctuations in the LFP during active (Figure 6.4A bottom, left) and quiet (Figure 6.4A bottom, right) bouts. The power spectrum of the raw LFP signal filtered for beta band activity during epochs of TJMs (red) and no TJMs (blue) revealed strong LFP beta band power, but no alterations in power across behavioral state (e.g., bouts of TJMs vs. no TJMs). At a simultaneously recorded STN site, the same pattern of activity was present (Figure 6.4B). Overall, the bandpass filtered LFP signal (15-30 Hz) and subsequent power spectrum of that signal during epochs of TJMs and quiescence revealed no differences in beta band power (Figure 6.4B).

Summary data from all animals demonstrate the same trend as represented in Figure 6.4A/B. Overall, M1 LFPs during TJM epochs (red) and no TJM epochs (blue) exhibited similar levels of beta band power (Figure 6.4C; $t(18) = 1.48, p > .05$). For STN, the same pattern of activity existed (Figure 6.4C, right; $t(18) = 0.65, p > .05$). Further, M1 exhibited higher beta band power during epochs of TJMs and no TJMs as compared to STN (TJM: $t(18) = 2.76, p < .05$; no TJM: $t(18) = 3.12, p < .05$). Moreover, TJM epochs exhibited lower centroid beta band frequency compared to no TJM epochs for both M1 and STN (Figure 6.4D; M1: $t(18) = -2.84, p < .05$; STN: $t(18) = -3.57, p < .05$, respectively). Further, beta band frequency was higher in M1 during TJM epochs compared to STN ($t(18) = 3.63, p < .05$). Alternatively, there was no difference in beta band frequency between M1 and STN during periods of quiescence ($t(18) = -0.47, p > .05$).

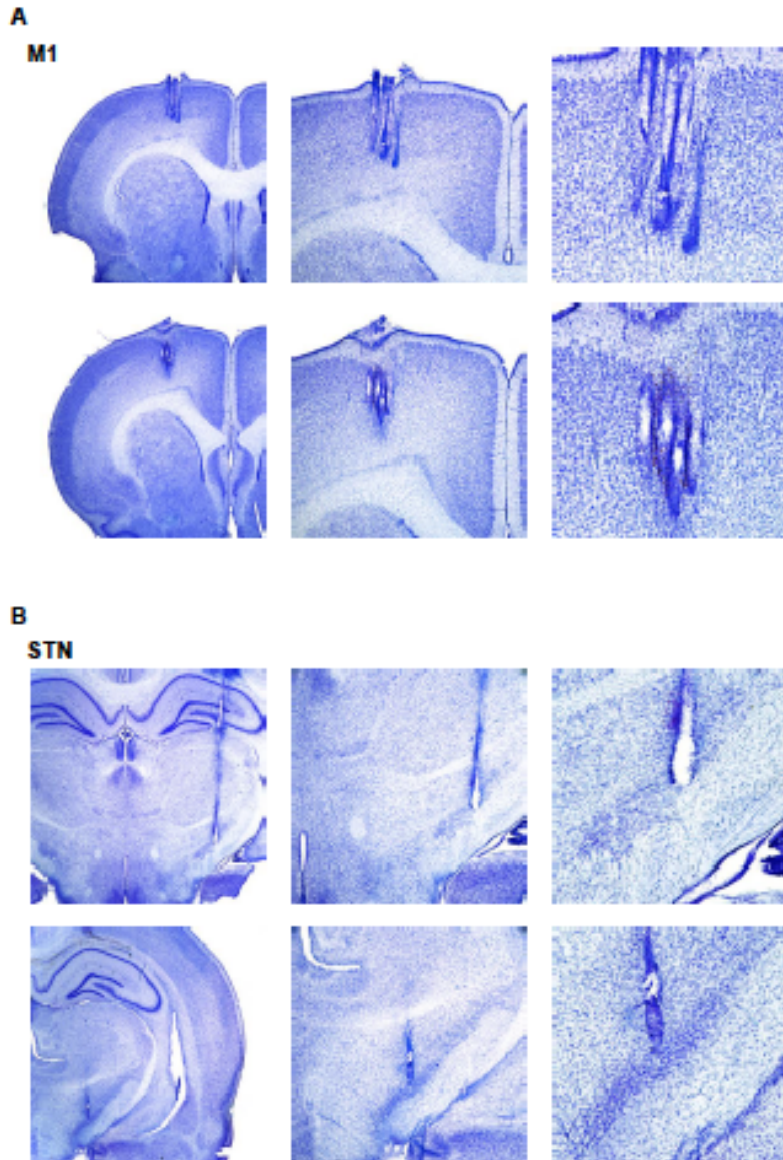


Figure 6.1: Verification of electrode placements. A (top): Photomicrographs of four representative and simultaneously recorded sites in M1. Middle and right photomicrographs show 4x and 10x close-up of electrode tips, respectively. A (bottom): Same as A (top) but for a different animal. B (top): Photomicrographs of a representative recording site in STN. Middle and right photomicrographs show a 4x and 10x close-up of electrode tips, respectively. B (bottom): Same as B (top) but for a different animal.

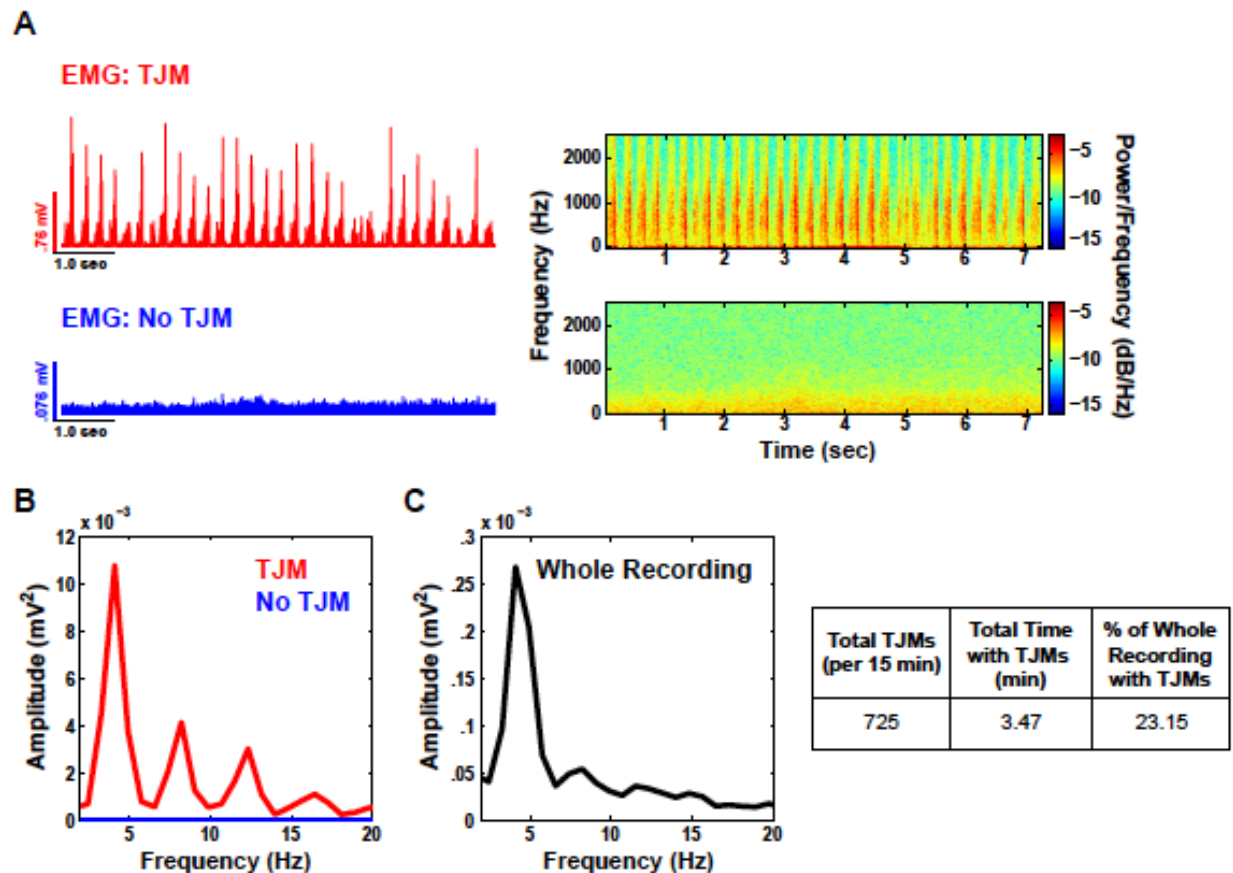


Figure 6.2: Pilocarpine induces TJMs in the tremor frequency range as reflected by EMG activity. A (top): EMG electrode trace bandpass filtered for EMG frequency (500-1500 Hz) and full wave rectified during a long epoch of TJMs (red; 7.3 seconds) for a representative animal. The spectrogram indicates the same pattern of rhythmic activity as the EMG trace. A (bottom): Same animal and recording as presented in A, but for a long period of quiescence (blue; 7.3 seconds). The spectrogram indicates little rhythmic activity during bouts lacking TJMs. B: Power spectrum of the EMG envelope for the same traces as presented in A. As can be seen, there is clear 3-7 Hz rhythmicity and strong harmonics during TJM epochs, which is absent during periods of quiescence. C (left): Power spectrum of the entire pilocarpine recording for the same animal as presented in A and B. C (right): Behavior of the same animal as presented previously across the entirety of the pilocarpine recording.

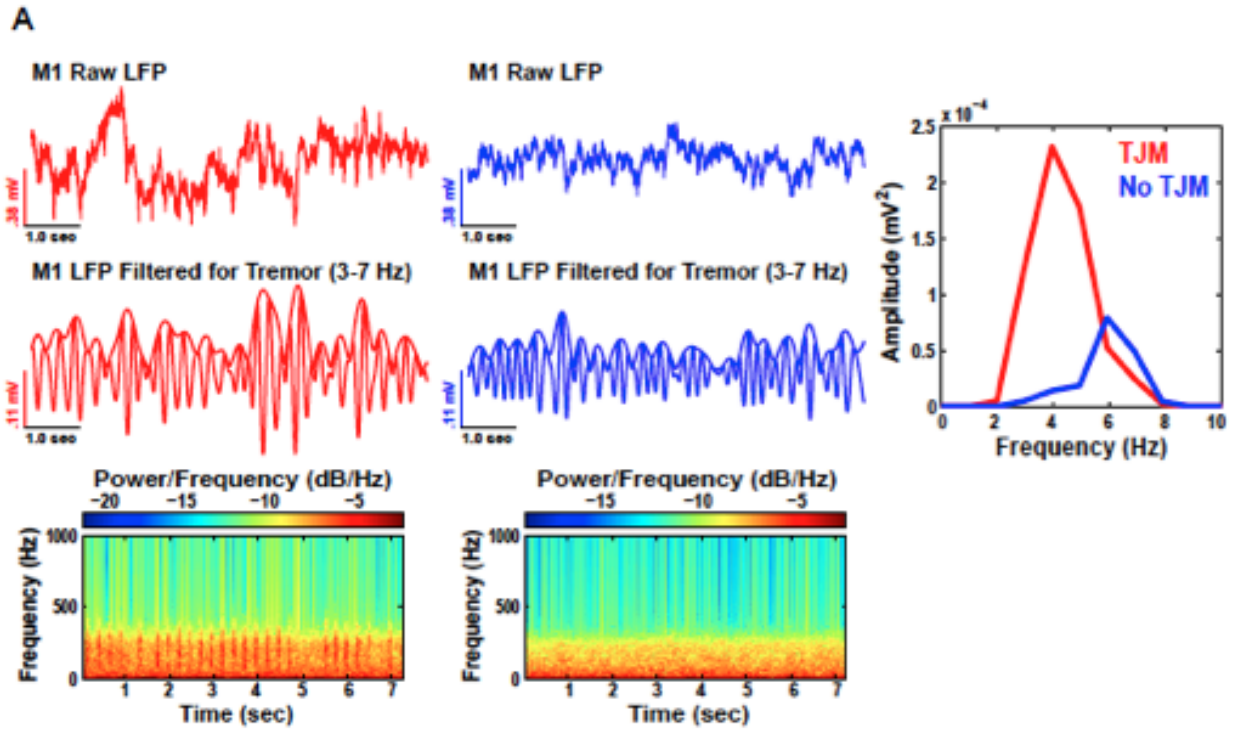


Figure 6.3A: Power in tremor frequency band increases during TJMs in M1. Top: Raw and bandpass filtered (3-7 Hz; middle) M1 LFP traces during epochs of TJMs (red) and no TJMs (blue) for the same representative animal presented in Figure 6.2. Bottom: Corresponding spectrograms for the raw M1 signal presented in the top panel during TJM and no TJM epochs. Overall, the spectrograms indicate rhythmicity in the LFP during periods of TJMs that is lacking during periods of quiescence. Right: Power spectrum of the bandpass filtered LFP signal presented in the middle panel during the TJM and no TJM epoch. Tremor frequency power increases substantially during periods of TJMs, while little tremor power exists during epochs of quiescence.

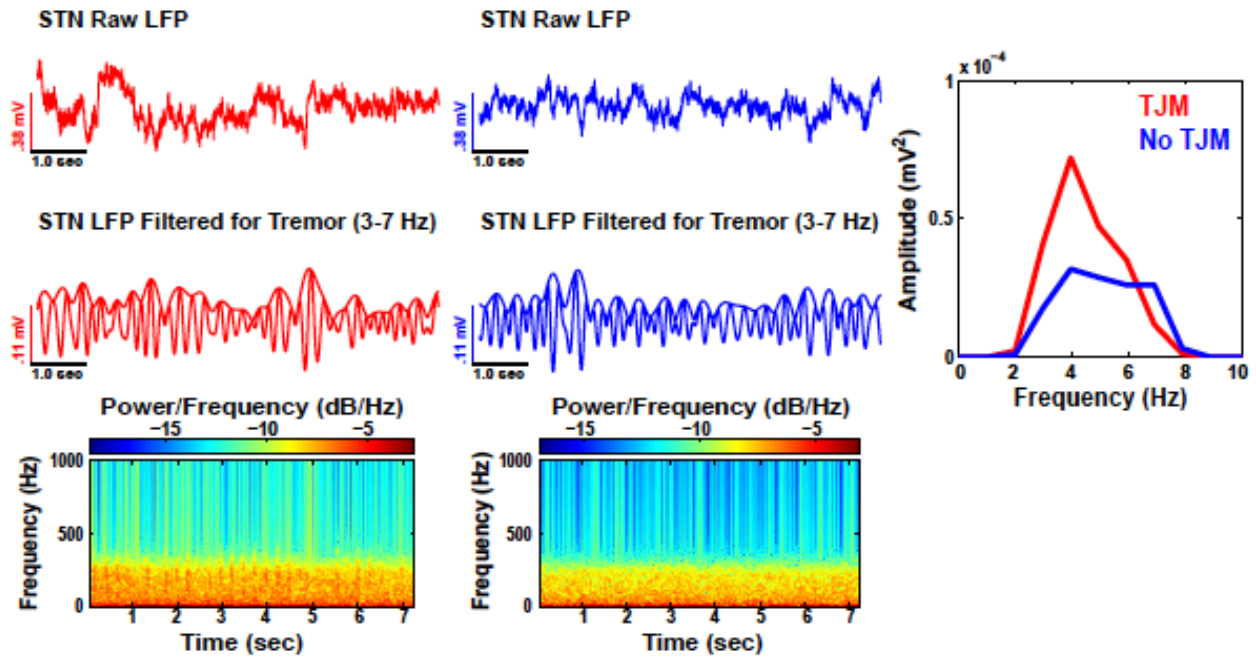
B

Figure 6.3B: Power in tremor frequency band increases during Tjms in STN. Top: Raw and bandpass filtered (3-7 Hz; middle) STN LFP traces during epochs of Tjms (red) and no Tjms (blue) for the same representative animal presented in Figure 6.2. Bottom: Corresponding spectrograms for the raw STN signal presented in the top panel during Tjms and no Tjms epochs. Overall, the spectrograms indicate rhythmicity in the LFP during periods of Tjms that is lacking during periods of quiescence. Right: Power spectrum of the bandpass filtered LFP signal presented in the middle panel during the Tjms and no Tjms epoch. Tremor frequency power increases substantially during periods of Tjms, while little tremor power exists during epochs of quiescence.

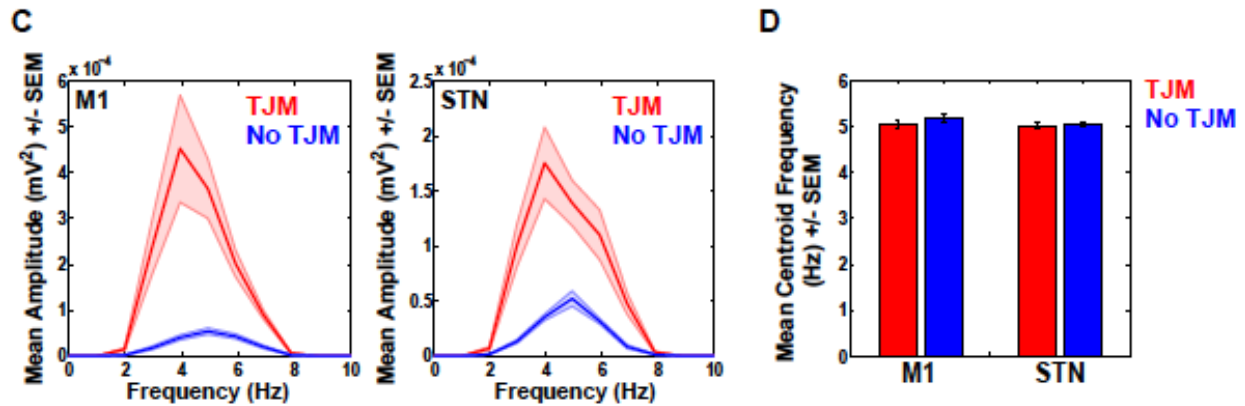


Figure 6.3 C and D: Summary data for tremor frequency power across all animals for TJM and no TJM epochs for M1 and STN. As can be seen, tremor band power dominates during the presence of TJMs, but not for bouts lacking TJMs. D: Summary data across all animals for tremor band centroid frequency as a function of TJM and no TJM epochs for M1 and STN recording sites. Overall, there were no differences in centroid frequency across behavioral state or brain area.

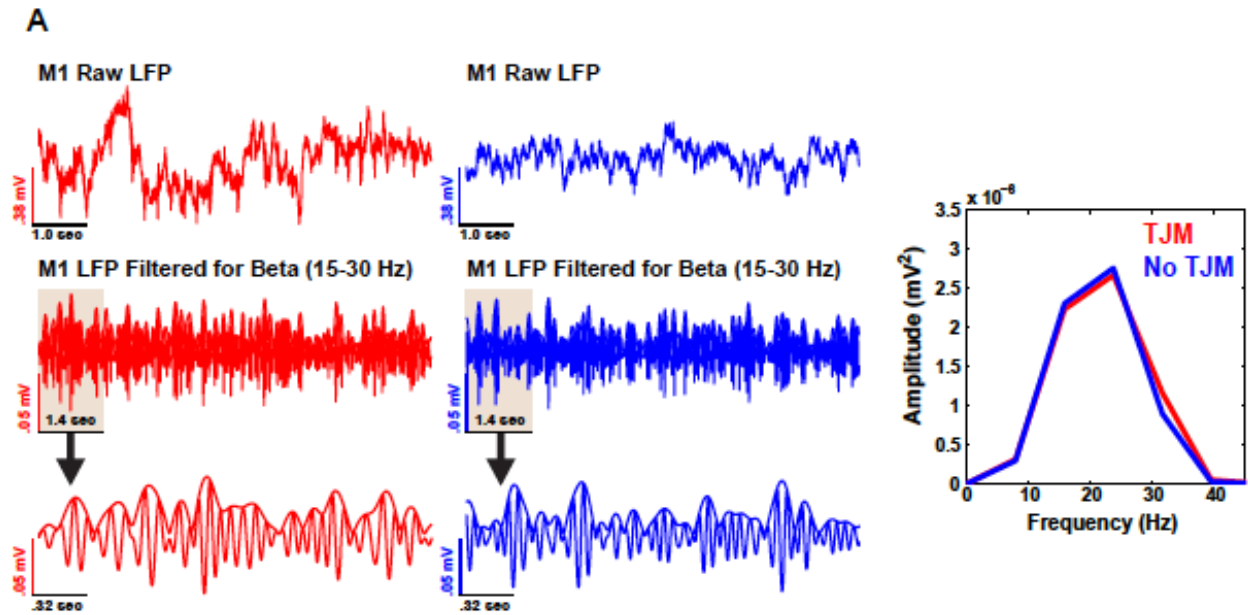


Figure 6.4A: Beta band power does not increase during TJMs in M1. Top: Raw and bandpass filtered (15-30 Hz; middle) M1 LFP traces during epochs of TJMs (red) and no TJMs (blue) for the same representative animal presented in Figure 6.2 and 6.3. Bottom: The first 1.4 seconds of the middle panel to show instantaneous LFP fluctuations. Right: Power spectrum of the bandpass filtered LFP signal presented in the middle panel during the TJM and no TJM epoch. Beta frequency power does not increase during periods of TJMs.

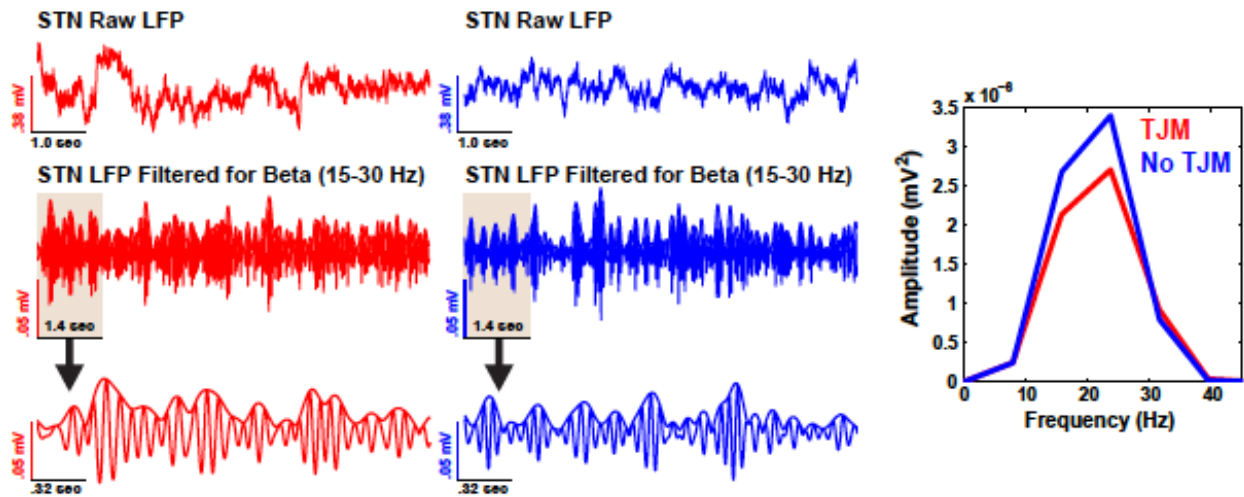
B

Figure 6.4B: Beta band power does not increase during TJMs in STN. Top: Raw and bandpass filtered (15-30 Hz; middle) STN LFP traces during epochs of TJMs (red) and no TJMs (blue) for the same representative animal presented in Figure 6.2 and 6.3. Bottom: The first 1.4 seconds of the middle panel to show instantaneous LFP fluctuations. Right: Power spectrum of the bandpass filtered LFP signal presented in the middle panel during the TJM and no TJM epoch. Beta frequency power does not increase during periods of TJMs.

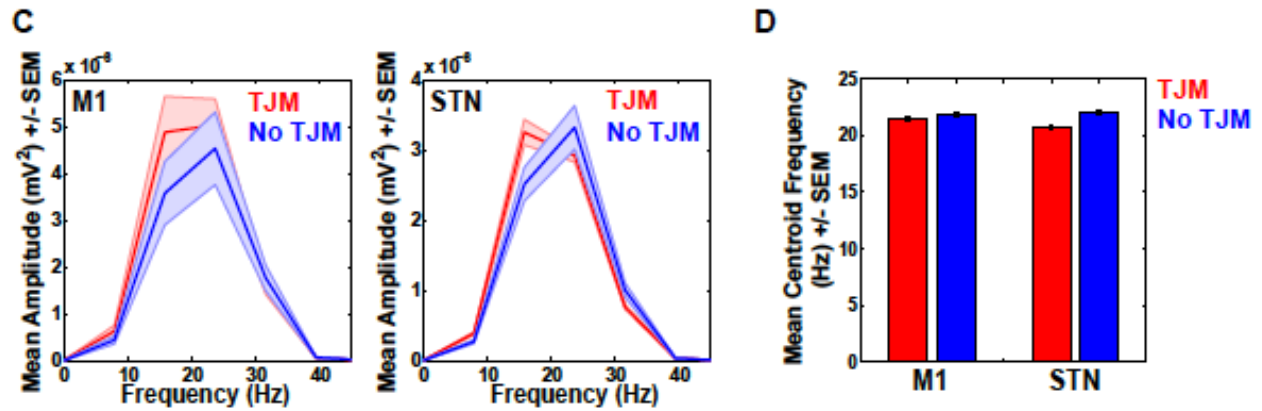


Figure 6.4 C and D: Beta band power does not increase during TJMs in M1 and STN. C: Summary data for beta band power across all animals for TJM and no TJM epochs for M1 and STN. As can be seen, beta band power is similar during across behavioral states. D: Summary data across all animals for beta band centroid frequency as a function of TJM and no TJM epochs for M1 and STN recording sites.

Chapter 7: Discussion

7.1 Summary of Results

The central aim of the present research was to characterize aspects of the instigation and treatment of Parkinsonian resting tremor using a pharmacological rodent model. These studies employed the TJM model, a rodent model of parkinsonian resting tremor, to investigate the neurochemical and electrophysiological changes that are associated with tremorogenesis. Furthermore, the TJM model was used to test the effects of both experimental and well established therapeutic agents. These studies collectively extended and validated the TJM model, contributing to the ultimate goal of this line of research, which is to characterize the conditions associated with tremorogenesis in order to develop specifically-targeted therapeutic strategies.

In the first set of experiments, administration of the anticholinesterase galantamine was shown to induce tremulous jaw movements in mice. Coadministration of the adenosine A_{2A} antagonist MSX-3 suppressed galantamine-induced TJMs. Freeze frame video analysis showed that the vast majority of galantamine-induced TJMs fell into the frequency range of 3-7.5 Hz, the frequency range characteristic of parkinsonian resting tremor. Coadministration of MSX-3 produced a slight alteration of the relative frequency distribution of galantamine-induced TJMs by slowing the peak local frequency from approximately 6 Hz to 5 Hz.

The second set of experiments evaluated the ability of safinamide to attenuate resting tremor induced by three drug treatments with different mechanisms of action. Safinamide is a reversible and selective MAO-B inhibitor that also reduces DA uptake, blocks voltage-dependent sodium channels, modulates N-type calcium channels, and reduces glutamate release. Safinamide significantly suppressed TJMs induced by the anticholinesterase galantamine and the

muscarinic agonist pilocarpine. Safinamide also attenuated the TJMs, but not the locomotor suppression, induced by subchronic administration of the DA D2 antagonist pimozide.

In the third group of experiments, the selective MAO-B inhibitor deprenyl suppressed TJMs induced by the VMAT-2 inhibitor tetrabenazine (TBZ). This study also employed *in vivo* microdialysis and HPLC to examine extracellular DA levels in the ventrolateral neostriatum (VLS). Administration of TBZ alone increased extracellular DA 30 minutes after injection, and reduced DA levels after 90 minutes compared to vehicle treated animals. Coadministration of deprenyl significantly increased extracellular DA in the later part of the session compared to rats treated with TBZ alone.

The fourth set of experiments was performed to characterize the effects of the antidepressant fluoxetine (FLX) on motor dysfunctions induced by the VMAT-2 inhibitor TBZ. In the TJM paradigm, a low dose of TBZ produced a small but significant increase in TJMs compared to vehicle control, and coadministration of TBZ and FLX produced a dramatic increase in TJMs compared to TBZ alone. Furthermore, administration of FLX decreased locomotion compared to vehicle control, and co-administration of TBZ plus FLX significantly decreased locomotion compared to TBZ alone. TJMs induced by coadministration of TBZ and FLX were significantly suppressed by administration of the 5-HT_{2A/2C} antagonist mianserin. Finally, DA tissue levels in the VLS were examined after administration of TBZ, FLX, and mianserin. TBZ administration decreased DA tissue levels in the VLS compared to the vehicle condition, and coadministration of TBZ and FLX further decreased DA tissue levels compared to TBZ alone. Mianserin attenuated this effect, as co-administration of mianserin in combination with TBZ and FLX increased DA tissue levels compared to the TBZ/FLX condition.

In the fifth set of experiments, LFPs from the primary motor cortex and subthalamic nucleus and EMG from the lateral temporalis muscle were simultaneously recorded in rats after administration of the muscarinic agonist pilocarpine. Pilocarpine administration induced TJMs that fell into the frequency range associated with Parkinsonian resting tremor (3-7 Hz). The induction of TJMs by pilocarpine was associated with strong rhythmicity in the envelope of the EMG signal with a peak frequency of approximately 4 Hz along with robust second and third harmonics. This EMG activity was accompanied by an increase in power at tremor frequency (3-7 Hz) in M1 and the STN. Tremor activity was not associated with increased activity in the beta frequency band.

7.2 Induction of oral tremor in mice by the acetylcholinesterase inhibitor galantamine:

Reversal with adenosine A_{2A} antagonism

The present studies investigated the ability of the anticholinesterase galantamine to induce TJMs in mice. The TJM model is a widely used model of Parkinsonian resting tremor that has been extensively validated in rats (Salamone et al., 1998, 2005, 2008b; Simola et al., 2004, 2006; Kasture et al., 2009; Trevitt et al., 2009). In order to provide cross-species validation of this model, researchers have made recent efforts to extend the TJM model to mice. Salamone et al. (2012) reported that administration of the muscarinic agonist pilocarpine induced TJMs in mice that fall into the frequency range of 3-7.5 Hz and can be attenuated by co-administration of the adenosine A_{2A} antagonist MSX-3, or by conditional neural knockout of the adenosine A_{2A} receptor. Furthermore, administration of the DA-depleting agent tetrabenazine induced TJMs in mice, which were attenuated by antagonism of the A_{2A} receptor with MSX-3, or by conditional neural knockout of the A_{2A} receptor (Podurgiel et al., 2013a).

Anticholinesterases are currently the primary treatment for cognitive dysfunction associated with Alzheimer's disease. Patients taking tacrine (Cognex), as well as the more recently developed anticholinergics such as donepezil, rivastigmine and galantamine, have been reported to experience improved cognition, but may develop Parkinsonian symptoms, including tremor, as a side effect (Ott and Lannon, 1992; Shea et al., 1998; Arai, 2000; Aarsland et al., 2003; Emre et al., 2004; Litvinenko et al., 2008; Song et al., 2008; Grace et al., 2009; van Laar et al., 2010). When administered to rats, galantamine induces TJMs that occur in the 3-7.5 Hz frequency range, and these movements were attenuated by co-administration of the adenosine A_{2A} antagonists MSX-3 and MSX-4 (Collins et al., 2011; Santerre et al., 2012). Consistent with these known tremorogenic properties of galantamine in rats, the present results show that galantamine significantly induces TJMs in mice in a dose-dependent manner (see Figure 2.1).

Galantamine-induced TJMs were significantly reduced by co-administration of the adenosine A_{2A} antagonist MSX-3 (see Figure 2.2). This observation is consistent with previous research showing that adenosine A_{2A} antagonists can produce antiparkinsonian effects in animal models (Wardas et al., 2001; Pinna et al., 2007; Morelli et al., 2010) as well as human studies (LeWitt et al., 2008; Mizuno and Kondo, 2013). Moreover, these results support the observation that adenosine A_{2A} receptor antagonism suppresses tremor in rodents (Correa et al., 2004; Simola et al., 2004, 2006; Tronci et al., 2007; Salamone et al., 2008a, 2013; Podurgiel et al., 2013a) and humans (Bara-Jimenez et al., 2003). The direct mechanism through which adenosine A_{2A} receptor antagonism interacts with cholinergic transmission to suppress tremorogenesis is unclear, however, there is considerable evidence indicating that the neostriatum is a critical site for the generation of TJMs in rats (Salamone et al., 1990, 1998, 2008a,b; Finn et al., 1997), and adenosine A_{2A} receptors are expressed in high concentrations in neostriatal enkephalin-positive

neurons (Ferré et al., 1997, 2001, 2008; Svenningsson et al., 1999; Chen et al., 2001; Fuxe et al., 2004). Furthermore, several studies in rats have shown that adenosine A_{2A} antagonism alters production of signal transduction markers such as cFos and pDARPP-32(Thr34) in putative or identified enkephalinergic striatal neurons (Betz et al., 2009; Santerre et al., 2012; Podurgiel et al., 2013a; Nunes et al., 2013). Thus, it is possible the adenosine A_{2A} antagonists such as MSX-3 are acting on enkephalin-positive striatopallidal neurons that form part of the so called “indirect pathway” in the basal ganglia circuitry, and that this action is capable of reducing the TJMs induced by DA antagonism and DA depletion (Betz et al., 2009; Podurgiel et al., 2013a), as well as cholinomimetic drugs.

Experiment 3 examined the local frequency of TJMs after administration of galantamine alone, and after co-administration of galantamine and MSX-3. Figure 2.3 shows the relative frequency distribution (i.e., expressed as a percent of total) of the inter-movement intervals for each TJM observed in the video analysis. The inter-movement interval is a useful measure because it is the reciprocal of the local frequency of movement (i.e., 200 ms inter-movement interval = 5 Hz). As hypothesized, the vast majority of galantamine-induced TJMs fell into the frequency range of 3-7.5 Hz. Although the neural circuitry that generates the underlying rhythmicity of TJMs is unknown, it does appear that in both rats and mice, the peak frequencies for TJMs induced by DA antagonism, DA depletion, muscarinic agonists and anticholinesterases all fall within the 3.0-7.5 Hz range, which is similar to that seen in Parkinsonian resting tremor (Salamone et al., 1998; Collins-Praino et al., 2011; Ishiwari et al., 2005; Podurgiel et al., 2013a). In experiment 3, co-administration of MSX-3 reduced the total number of TJMs, as seen in experiment 2. In addition, MSX-3 produced a slight alteration of the relative frequency distribution of galantamine-induced TJMs (i.e., as indexed by the significant bin x drug treatment

interaction), apparently by slowing the peak local frequency from approximately 6 Hz (bin 5 = 5/30 sec inter-movement interval) to 5 Hz (bin 6 = 6/30 sec inter-movement interval).

Taken together, these results establish a mouse model of cholinomimetic-induced TJMs using the anticholinesterase galantamine, and thereby give researchers an additional avenue for investigating drug-induced Parkinsonism and tremorogenesis. Moreover, these results strengthen the literature demonstrating that adenosine A_{2A} antagonists have anti-tremor effects. Future studies should examine the effects of galantamine administration on tremor generation in mice with various genetic manipulations that are related to Parkinsonism.

7.3 Tremorolytic effects of safinamide in animal models of drug-induced parkinsonian tremor

The studies presented in chapter 3 were conducted to determine if safinamide is capable of reversing the oral tremor induced by galantamine, pilocarpine, and pimozide, using the well established tremulous jaw movement model. The results described above demonstrate that safinamide suppressed tremor in all three experiments, which to our knowledge is the first demonstration of the tremorolytic effects of safinamide. In the first experiment, all seven doses of safinamide tested (0.312 mg/kg to 10.0 mg/kg) were capable of reversing galantamine-induced tremulous jaw movements. In the second experiment, three of the seven doses of safinamide tested (0.625 mg/kg, 5.0 mg/kg, 10.0 mg/kg) were able to attenuate tremulous jaw movements induced by pilocarpine. In the final experiment, both tremulous jaw movements and locomotion were assessed as in the Salamone et al. (2008a) paper. In that study, it was shown that subchronic administration of 1.0 mg/kg of the DA D2 antagonist pimozide induced tremulous jaw movements and reduced locomotion, relative to vehicle-treated rats. Three of the

seven doses tested (1.25 mg/kg, 5.0 mg/kg, 10.0 mg/kg) were capable of reducing pimozone-induced tremulous jaw movements. Thus, across three different drug treatments, which have different mechanisms of action and generate different levels of tremor activity, safinamide was effective at reducing tremor. In the present study, although safinamide consistently attenuated tremulous jaw movements, it did not reverse the locomotion suppression induced by pimozone. It is not clear why safinamide failed to reverse the suppression of locomotion induced by pimozone, because other putative antiparkinsonian drugs, such as adenosine A_{2A} antagonists, are capable of inducing locomotor activity in pimozone-treated animals at doses that also reduce tremor (Salamone et al. 2008a). It is possible that safinamide is particularly effective at reducing tremor because of some specific neurochemical actions of the compound, but this remains to be determined.

The ability of safinamide to reverse tremulous jaw movements induced by galantamine, pilocarpine, and pimozone is consistent with previous animal research demonstrating the antiparkinsonian properties of safinamide (Salvati et al. 1999; Fariello et al. 2000; Onofrj et al. 2008 Schapira 2010). Additionally, our results are consistent with data from clinical studies. In the few published studies on the efficacy of safinamide in Parkinson's disease patients, results show that safinamide is capable of treating motor impairments (Stocchi et al. 2004, 2006; Onofrj et al. 2008; Schapira 2010). When administered to early Parkinson's disease patients, 37.5% revealed a 30% or greater improvement in the Unified Parkinson's Disease Rating Scales (UPDRS III) score at the end of the study versus baseline (Stocchi et al. 2004). Additionally, when administered to Parkinson's disease patients as an add-on therapy to a DA agonist, 47.1% showed improvement (Stocchi et al. 2004).

The mechanism of action through which safinamide acts to suppress parkinsonian

symptoms in humans, or drug-induced tremors in rats, is uncertain. As described above, safinamide is an inhibitor of MAO-B, and also blocks DA uptake, effects that lead to an elevation of extracellular DA (Marzo et al. 2004; Fariello 2007; Binda et al. 2007; Onofrj et al. 2008; Schapira 2010; Stocchi 2012). However, non-dopaminergic strategies also are becoming increasingly important in the development of drug treatments for parkinsonian symptoms. Neostriatal DA interacts with several other transmitters in the circuitry of the basal ganglia, including glutamate, GABA, serotonin, adenosine, and acetylcholine (DeLong, 1990; Hauber, 1998; Obeso et al. 2000; Young and Penney, 1993). Thus, it is possible that some of the non-dopaminergic actions of safinamide, including effects on sodium channels, calcium channels, and glutamate release, also would lead to antiparkinsonian effects (Onofrj et al. 2008; Schapira 2010; Stocchi 2012). Safinamide has anticonvulsant properties (Fariello et al. 1998), and it has been suggested that there are fundamental similarities between the physiology that underlies the production of seizures and tremorogenesis (Buzsaki et al. 1990; Salamone et al. 1998). Further research will be necessary to elucidate the specific mechanism through which safinamide exerts its effects on motor dysfunctions, including tremor, which are related to parkinsonism.

7.4 MAO-B inhibition attenuates Parkinsonism induced by the VMAT-2 inhibitor tetrabenazine

The studies in chapter 4 were performed to determine the effect of administration of the selective MAO-B inhibitor deprenyl on the TJMs induced by the VMAT-2 inhibitor tetrabenazine in rats. Our lab has previously established a pharmacological rodent model of Parkinsonism using TBZ, showing that TBZ administration induces tremulous jaw movements, catalepsy, and locomotor suppression in rats (Podurgiel et al., 2013a). These motor abnormalities

can be attenuated by coadministration of the adenosine A_{2A} antagonist MSX-3, a putative antiparkinsonian agent (Podurgiel et al., 2013a). Until the present work, MSX-3 had been the only antiparkinsonian agent used to assess TBZ-induced TJMs. Given the importance of validating animal models, experiment 1 examined the ability of the well-established antiparkinsonian agent deprenyl to suppress tremor induced by TBZ. While MSX-3 exerts its antiparkinsonian properties via antagonism of adenosine A_{2A} receptors, deprenyl acts to selectively inhibit MAO-B, one of the primary enzymes responsible for DA metabolism in the brain (Youdim and Bakhle, 2006). As predicted, results from experiment 1 showed that administration deprenyl significantly attenuated TBZ-induced TJMs. These results are consistent with clinical reports demonstrating the antiparkinsonian properties of deprenyl (Schapira et al., 2011; Riederer P, Laux G, 2011; Fabbrini et al., 2012) and provide validation for the use of TBZ as a rodent model for assessment of drugs, as researchers have now shown that TBZ-induced Parkinsonism can be attenuated by two antiparkinsonian agents with different mechanisms of action, an adenosine A_{2A} antagonist (Podurgiel et al., 2013a) and a MAO-B inhibitor. Furthermore, this experiment is the first to show that a selective MAO-B inhibitor is capable of attenuating TJMs in rodents. A previous study from our lab has shown that safinamide is capable of attenuating TJMs induced by a muscarinic agonist, anticholinesterase, and dopamine antagonist (Podurgiel et al., 2013b; Chapter 3). While safinamide does reversibly inhibit MAO-B, it also reduces dopamine uptake, blocks voltage-dependent sodium channels, modulates N-type calcium channels, and reduces glutamate release (Marzo et al., 2004; Onofri et al., 2008).

The second experiment examined extracellular DA levels in the ventrolateral striatum (VLS), after administration of TBZ alone, and coadministration of TBZ and deprenyl. The VLS is the neostriatal area most closely related to the production of TJMs, as it has been shown to be

responsible for orofacial movements and forepaw motor control (Salamone et al., 1990; Jicha and Salamone, 1991; Salamone et al., 1993). Previous work has demonstrated that TJMs induced by the DA D2 antagonist pimozone could be attenuated by local injections of MSX-3 into the VLS (Salamone et al., 2008a). Results from experiment 2 showed that administration of TBZ alone resulted in a substantial increase (100%) in extracellular DA 30 minutes after injection, followed by a significant reduction (50%) after 90 minutes compared to vehicle treated animals. This biphasic effect of TBZ on striatal DA is consistent with previous literature, and this early, short-lived increase in extracellular DA can likely be attributed to an initial build-up of intracellular DA due to a blockade of storage, and possibly a subsequent reversal of the DA transporter (Andersson 2006). It is reasonable to suggest that as time goes by, intracellular MAO has degraded a substantial percentage of the unstored cytosolic DA molecules, resulting in the observed decrease in extracellular DA, which is maintained for 60 minutes. Consistent with the behavioral data in experiment 1, coadministration of deprenyl blunted the neurochemical actions of TBZ, as deprenyl significantly increased extracellular DA compared to rats treated with TBZ alone in the latter part of the microdialysis session. It is likely that this occurred because inhibition of MAO-B by deprenyl would have prevented the enzymatic breakdown of the cytosolic, non-vesicular fraction of the DA pool.

Taken together, these results provide evidence validating the use of TBZ as a rodent model of drug-induced Parkinsonism. Future studies should continue to validate and utilize this model to examine putative antiparkinsonian agents, and provide further insight into the mechanisms associated with tremorogenesis.

7.5 Fluoxetine administration exacerbates oral tremor and striatal dopamine depletion in a rodent pharmacological model of Parkinsonism

The present studies were undertaken to characterize the effects of the antidepressant FLX on motor dysfunctions, particularly the oral tremor marked by TJMs, which are induced by the VMAT-2 inhibitor TBZ. These drugs were selected because Huntington's disease patients prescribed TBZ for the treatment of chorea may experience Parkinsonism and depression as adverse events (Kenney et al., 2007; Frank, 2009; Guay, 2010), and also because SSRIs such as FLX are frequently used to treat depression associated with Parkinsonism (Veazey et al., 2005; Aarsland et al., 2009; Chen and Marsh, 2013; Schreiber and Thompson, 2013). Administration of high doses of TBZ (e.g. 2.0 mg/kg) in rats has been used as a pharmacological rodent model of Parkinsonism (Podurgiel et al., 2013a), while lower doses such as 0.75 mg/kg are used to model motivational symptoms of depression (Nunes et al., 2013; Randall et al., 2014). Experiment 1 showed that a low dose of TBZ (0.75 mg/kg) produced a small but significant increase in TJMs compared to the vehicle condition, and this effect of TBZ was dramatically enhanced by co-administration of FLX (2.5, 5.0, or 10.0 mg/kg), which significantly increased TJMs compared to TBZ alone. Additionally, administration of FLX (10.0 mg/kg) significantly decreased locomotion compared to vehicle control, and co-administration of TBZ (0.75 mg/kg) plus FLX (2.5, 5.0, 10.0 mg/kg) significantly decreased locomotion compared to TBZ alone. Thus, results from experiment 1 showed that FLX administration exacerbates Parkinsonian-like motor dysfunctions induced by a low dose of TBZ in rats. These results are consistent with studies showing potentiation of haloperidol-induced motor impairments (e.g. catalepsy and bradykinesia) by FLX in rodents (Tatara et al., 2012), and with numerous clinical reports linking

motor side effects and worsening Parkinsonism with FLX treatment of depression (Leo, 1996; Gerber and Lynd, 1998; Govoni et al., 2001; Madhusoodanan et al., 2010).

Previous research has demonstrated that 5-HT is involved in the modulation of drug and lesion-induced TJMs. Stewart et al. (1987) reported that pilocarpine-induced vacuous chewing-like movements were reduced by co-administration of para-chlorophenylalanine, which blocks 5-HT synthesis and depletes central 5-HT stores. Evidence also indicates that 5-HT₂ family receptors are involved in the regulation of TJM activity. Atypical antipsychotic drugs that act as 5-HT₂ family antagonists or inverse agonists generally fail to induce TJMs, and in fact can suppress the TJMs induced by cholinomimetic drugs (Salamone et al., 1998; Betz et al., 2005, 2009). Furthermore, the 5-HT_{2A/2C} receptor antagonist mianserin was shown to suppress the TJMs induced by the 5-HT agonists m-chlorophenylpiperazine and quipazine (Stewart et al., 1989), as well as anticholinesterase tacrine (Carlson et al., 2003). In view of these previous findings, experiment 2 investigated the ability of mianserin to attenuate the TJMs induced by co-administration of TBZ (0.75 mg/kg) and FLX (5.0 mg/kg). The results showed that administration of mianserin (2.5, 5.0 mg/kg) significantly reduced the number of TJMs induced by co-administration of TBZ and FLX, thereby indicating that FLX exacerbates TJMs via activation of 5-HT_{2A} and/or 5-HT_{2C} receptors. Although mianserin is nonselective for different subtypes of 5-HT₂ receptors, previous work has shown that the rank order of potency for the suppression of tacrine-induced TJMs by atypical antipsychotics is related to their affinity for 5-HT_{2A} receptors (Betz et al., 2005), and also that the selective 5-HT_{2A} inverse agonist ACP-103 could reduce tacrine-induced TJMs (Vanover et al., 2008). Future studies should investigate the effects of selective antagonists or inverse agonists of 5-HT_{2A} and 5-HT_{2C} receptors on the fluoxetine-induced enhancement of tetrabenazine-induced TJMs.

Previous research has shown that the VLS is the neostriatal region most closely associated with the production of TJMs (Kelley et al., 1989; Salamone et al., 1990, 1998, 2008; Jicha and Salamone, 1991; Betz et al., 2009), and that DA depletion in the VLS, but not other striatal subregions, induces TJMs in rats (Jicha and Salamone, 1991). Therefore, in order to determine if changes in striatal DA levels are related to the pattern of behavioral effects observed in experiments 1 and 2, experiment 3 examined DA tissue levels in the VLS of rats after administration of TBZ (0.75 mg/kg), FLX (5.0 mg/kg) and mianserin (5.0 mg/kg). As predicted, TBZ administration decreased DA tissue levels in the VLS compared to the vehicle condition. Consistent with the pattern of effects observed in the behavioral experiments, co-administration of TBZ and FLX further decreased DA tissue levels compared to TBZ alone, and mianserin attenuated this effect, with co-administration of mianserin in combination with TBZ and FLX increasing DA tissue levels compared to the TBZ/FLX condition.

The precise mechanisms through which FLX enhances the TJMs induced by TBZ is not clear. Although one cannot completely discount the possibility of specific pharmacodynamic or pharmacokinetic interactions between these two particular drugs, it is important to emphasize that motor dysfunctions induced by FLX or other 5-HT uptake inhibitors have been reported to occur in humans and other animals under conditions in which a variety of other drugs were being administered, and even when no other drugs were being given. Furthermore, the ability of mianserin to reduce TJMs has been observed under a variety of conditions, including cases in which either a 5-HT agonist (Stewart et al., 1989) or an anticholinesterase (Carlson et al., 2003) was being administered. The present results are consistent with a substantial literature demonstrating serotonergic modulation of TJMs across a broad array of pharmacological conditions (Stewart et al., 1987, 1989; Salamone et al., 1998; Carlson et al., 2003; Betz et al.,

2007; Vanover et al., 2008). Serotonergic neurons from the raphe nucleus project to the dopaminergic midbrain nuclei (substantia nigra pars compacta and ventral tegmental area), as well as to their target structures, the striatum and nucleus accumbens (Michelsen et al., 2007). The 5-HT₂ receptor family, with particular emphasis on the 5-HT_{2A} and 5-HT_{2C} receptor subtypes, has been implicated in serotonergic modulation of mesolimbic and nigrostriatal DA activity (Di Giovanni et al., 1999; Di Matteo et al., 2000; Porras et al., 2002; Alex et al., 2005; Alex and Pehek, 2007), though it is unclear how these physiological interactions are related to the present findings. Prototypical SSRIs like FLX block uptake of 5-HT from the synapse by inhibiting the 5-HT transporter, resulting in increased synaptic levels of 5-HT and increased activation of 5-HT receptors (Nutt et al., 1999). Furthermore, FLX treatment has been shown to reduce levels of DA and its metabolite in the neostriatum of mice (Morelli et al., 2011). Based upon the present results, it is reasonable to hypothesize that FLX administration may be enhancing the TJMs induced by TBZ by reducing DA tissue levels in VLS, an effect that is mediated via increased stimulation of 5-HT_{2A} and/or 5-HT_{2C} receptors. Nevertheless, interactions involving other brain areas (e.g. substantia nigra) and neurotransmitters (e.g. acetylcholine) may also be important.

Overall, the results from this study indicate that acute FLX administration exacerbates drug-induced Parkinsonism in a pharmacological rodent model, possibly due to increased stimulation of 5-HT_{2A} and/or 5-HT_{2C} receptors. Since antidepressant drugs are typically given chronically, future studies should also examine the effects of long-term administration of FLX and other SSRIs. These results have significant implications for understanding the movement-related adverse events, including tremor, which are induced by FLX (Brambilla et al., 2005; Madhusoodanan et al., 2010), and the complications that may result from the continued use of

FLX in patients with idiopathic or drug-induced Parkinsonism, particularly in view of evidence suggesting that SSRIs may be no more effective than placebo in treating PD-related to depression (Skapinakis et al., 2010; Aarsland et al., 2009).

7.6 Tremor-related subthalamic and cortical local field potentials associated with pilocarpine-induced oral tremor

Previous research has shown that the muscarinic agonist pilocarpine is a tremorogenic agent. Pilocarpine induces a robust TJM response in the 3-7 Hz frequency range that is reduced by antiparkinsonian agents (Salamone et al., 2005; Betz et al., 2007; Collins et al., 2010; Podurgiel et al., 2013b), conditional neural knockout of adenosine A_{2A} receptors (Salamone et al., 2013), and deep brain stimulation of the STN (Collins-Praino et al., 2013). Evidence indicates that the TJMs induced by pilocarpine are due to stimulation of M2 or M4 muscarinic receptors in the ventrolateral neostriatum of the rat, which is the homologue of the ventral putamen of primates (Salamone et al., 1990, 1998; Mayorga et al., 1997, 1999). For these reasons, pilocarpine was selected for the present studies in order to induce a robust oral tremor that would allow for the assessment of tremor-related cortical and subthalamic LFP activity.

Network activation as measured by LFP activity in M1 and STN can be used as a tool to better understand transient dynamics across distributed neural networks. Similar to analysis of variations in the blood-oxygen-dependent signal used in functional neuroimaging (Logothetis and Wandell, 2004; Law et al., 2005), detailed analysis of mesoscopic signals such as the LFP and EEG reveal the engagement of distributed neural circuits in relation to tremorogenesis—a cardinal symptom of Parkinsonism. Abnormalities in long-range connectivity between brain areas have been postulated as an important pathophysiological mechanism underlying brain

dysfunctions (Hutchison et al., 2004; Mallet et al., 2008). However, it remains unclear how perturbed connectivity relates to motor symptoms such as tremorogenesis, and how it is manifested in the dynamic interactions of neuronal circuits.

In the present study, we simultaneously recorded LFPs from the primary motor cortex and subthalamic nucleus as well as EMG from the lateral temporalis muscle in a rat model of drug-induced tremor. The temporalis was chosen because of previous research indicating that activity of this jaw closing muscle is a critical marker of observable TJM activity (Cousins et al., 1998). The present results indicate that administration of the muscarinic agonist pilocarpine induces TJMs in rats that fall into the frequency range associated with Parkinsonian resting tremor (3-7 Hz), with a peak frequency of approximately 4 Hz, and harmonics at higher frequencies. These data are consistent with previous studies indicating that cholinomimetic drugs, including muscarinic agonists and anticholinesterases, can induce or exacerbate resting tremor. Administration of the anticholinesterase physostigmine to PD patients was reported to exacerbate parkinsonian symptoms, including tremor, and these motor deficits were attenuated by coadministration of centrally-acting muscarinic antagonists (Duvoisin 1967). For many decades, non-selective muscarinic receptor antagonists have been used to treat idiopathic and drug-induced Parkinsonism (McEvoy, 1983). Anticholinesterases are prescribed to treat the cognitive deficits associated with Alzheimer's Disease (see Birks, 2006 for review), and these drugs have been shown to induce Parkinsonian symptoms, including tremor, as side effects (Ott and Lannon, 1992; Arai, 2000; Aarsland et al., 2003; Grace et al., 2009). In animal studies, muscarinic agonists such as tremorine and oxotremorine have been widely recognized to act as tremorogenic agents (Brimblecombe, 1975). Furthermore, muscarinic agonists and anticholinesterases induce TJMs in rodents, and co-administration of antiparkinsonian agents

including DA agonists, muscarinic antagonists, and adenosine A_{2A} antagonists have been shown to reduce cholinomimetic-induced TJMs (Salamone et al. 1986; Baskin et al., 1994; Mayorga et al., 1997; Salamone et al., 1998; Simola et al., 2004, 2006; Miwa et al., 2009; Collins et al., 2010a, 2011).

The induction of TJMs by pilocarpine was associated with strong rhythmicity in the envelope of the EMG signal with a peak frequency of approximately 4 Hz along with robust second and third harmonics, which is consistent with EMG recordings from the forearms of PD patients during periods of tremor (Liu et al., 2002; Timmerman et al., 2003; Wang et al., 2005; Reck et al., 2009; Hirschmann et al., 2013). This EMG activity was accompanied by an increase in power at tremor frequency (3-7 Hz) in M1 and the STN. In PD patients, pathological oscillatory neuronal activity in the open and closed loop connections between the cortex, basal ganglia, and thalamus is thought to underlie tremorogenesis (Hutchison et al., 2004). Simultaneously recorded magnetoencephalography and forearm EMG in PD patients has allowed researchers to characterize the cortical regions that are coherent with muscle activity during periods of resting tremor (Oswal et al., 2013). These studies indicate a strong coherence between EMG of forearm muscles and M1 activity at tremor frequency and its second harmonic (Timmerman et al., 2003).

Oscillatory activity in the STN of PD patients has been well characterized, as researchers are able to record LFPs from patients undergoing implantation of stimulating electrodes for deep brain stimulation. In recent years, the primary focus has been on increased oscillatory activity in the beta band (~15-30 Hz), since there is evidence that increased beta power in the STN is associated with motor control, particularly akinesia-rigidity (Brown et al., 2001; Levy et al., 2002; Priori et al., 2004; Brown and Williams, 2005; Kuhn et al., 2006). Conversely, resting

tremor has not been shown to correlate with beta band activity in the STN of PD patients (Kuhn et al., 2005). Instead, the development of tremor has been associated with the emergence of oscillations in the tremor frequency range (3-7 Hz) as indicated by power spectra of STN LFPs, and coherence between STN LFPs and EMG activity at tremor frequency (Brown et al., 2001; Levy et al., 2000; Liu et al., 2002; Wang et al., 2005; Reck et al., 2009; Hirschmann et al., 2013). In a recent study by Hirschmann et al. (2013) the emergence of tremor in PD patients was shown to be associated with an increase of cerebral synchronization at tremor frequency and second harmonic in a network that includes both STN and M1. Additionally, in African green monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the development of resting tremor was associated with the emergence of oscillations at tremor frequency in the STN (Bergman et al., 1994). Results from our experiment are line with the findings reported in the clinical and non-human primate studies, as we saw increased power in M1 and STN in the tremor, but not beta frequency range during periods of TJMs. Moreover, the present results are consistent with previous studies demonstrating that the STN is a critical part of the basal ganglia circuitry that is involved in motor dysfunctions related to Parkinsonism. Lesions or inactivation of STN have been shown to reverse motor dysfunctions in rodent models (Centonze et al., 2005; Baunez and Gubellini 2010). In addition, high frequency stimulation of STN has been reported to restore motor function in rodent models of Parkinsonism (Baunez 2011; Brown et al., 2011), and to attenuate drug-induced TJMs in rats (Collins-Praino et al., 2011).

To date, this phenomenon has been well documented in the clinical literature, but very little has been done to study these oscillatory alterations in rodent models. It has, however, been shown that LFPs recorded from the frontal cortex and STN of rats with 6-hydroxydopamine lesions of midbrain dopaminergic neurons show increased power and coherence in the beta

frequency band (Sharott et al., 2005; Mallet et al., 2008). It should be noted that these studies were using a model that involved neurotoxic depletion of DA, whereas our study employed a model of drug-induced Parkinsonian resting tremor. In the present study, though the rats appeared to have reduced locomotion after pilocarpine administration, they were not completely akinetic, and occasionally moved about the chamber during recording. Therefore, our study specifically evaluated the physiological correlates of tremor by using an agent that induces a robust tremorogenic (i.e., TJM) response. By providing physiological and behavioral correlates of tremor, this model could be utilized in preclinical studies focused on the development of treatments that specifically target tremor.

7.7 Conclusions and Future Directions

The findings in the present research provide insight into aspects of the generation, maintenance, and treatment of Parkinsonian resting tremor. Experiment 1 established a mouse model of cholinomimetic-induced TJMs using the anticholinesterase galantamine, which gives researchers an additional platform for investigating drug-induced Parkinsonism and tremorogenesis. Results from this experiment also lend support the literature demonstrating the anti-tremor effects of adenosine A_{2A} antagonists. Future studies should utilize this model to examine the effects of galantamine administration on tremor generation in mice with various genetic manipulations that are related to Parkinsonism.

Results from experiment 2 support the use of safinamide as an adjunct to levodopa or DA agonists in PD patients, as it was effective in suppressing tremor induced by three different drug treatments with varying mechanisms of action. However, the precise mechanism of action through which safinamide acts to suppress Parkinsonian symptoms is uncertain. While it is a

potent, highly selective and reversible inhibitor of MAO-B, safinamide also reduces DA uptake, blocks voltage-dependent sodium channels, modulates N-type calcium channels, and reduces glutamate release. Further research is necessary to elucidate the specific mechanism through which safinamide exerts its effects on motor dysfunctions related to Parkinsonism.

In experiment 3, coadministration of the MAO-B inhibitor deprenyl blunted the number of TJMs and the neurochemical actions induced by administration of the VMAT-2 inhibitor tetrabenazine. These results are consistent with the use of deprenyl for the treatment of the motor symptoms of PD, and provide additional validation for the use of TBZ as a rodent model of Parkinsonism. Future studies should continue to validate and utilize this model to examine the mechanisms associated with tremor generation, and evaluate potential antiparkinsonian agents. Furthermore, because relatively high doses of deprenyl were used in experiment 3, it is possible that effects on MAO-A could have contributed to the behavioral and neurochemical effects that were seen. Thus, future studies should try to determine if the results reported above were due to selective actions of deprenyl on MAO-B, or a combined effect of blockade of both isozymes.

Results from experiment 4 indicate that acute FLX administration exacerbates drug-induced Parkinsonism in a pharmacological rodent model, possibly due to increased stimulation of 5-HT_{2A} and/or 5-HT_{2C} receptors. These results have significant implications for the continued use of FLX in patients with idiopathic or drug-induced Parkinsonism, particularly in view of clinical evidence suggesting that SSRIs may be no more effective than placebo in treating PD-related to depression. Results from this study contribute to the overall understanding of the movement-related adverse events that are induced by FLX, but since antidepressant drugs are typically given chronically, future studies should examine the effects of long-term administration of FLX and other SSRIs.

Results from experiment 5 parallel the findings reported in the clinical literature. In PD patients, the development of tremor has been shown to be associated with the emergence of oscillations in the tremor frequency range (3-7 Hz) in the cortex and subthalamic nucleus. While this phenomenon has been well characterized in PD patients, very little has been done to study these oscillatory alterations in rodent models. By providing physiological and behavioral correlates of tremor, this study establishes a model that could be utilized in preclinical studies focused on the development of treatments that specifically target tremor.

Collectively, these studies provide extensive validation for the TJM model. Future research should continue to utilize the TJM model to further investigate the pathophysiological mechanisms associated with tremor generation, as well as to evaluate novel therapeutics for the treatment of this motor symptom. The results presented here help to characterize the conditions associated with tremorogenesis, and can hopefully contribute to the development of specifically-targeted therapeutic strategies for the millions of patients suffering from Parkinsonism.

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