

## **Dopamine/Adenosine Interactions in an Animal Model of Parkinsonian Tremor**

Parkinson's Disease (PD) is currently the second most prevalent neurodegenerative disorder, with 1.5 million cases in the United States alone. PD, however, is just one member of a broader class of motor disorders termed "parkinsonism," whose hallmark symptoms include akinesia (lack of movement), bradykinesia (slowed movement), rigidity, and tremor. Little is currently known about the neural mechanisms involved in tremorogenesis. Since tremor is often the first symptom seen in parkinsonian individuals, and is very debilitating, there is a growing interest in investigating these mechanisms, as well as novel treatments for this symptom.

To date, this research has focused on several neurotransmitter systems in the basal ganglia, particularly the interactions among dopamine, adenosine, and acetylcholine. Recent studies have demonstrated the possible relevance adenosine A2A antagonists may have in treating parkinsonian symptoms [1]. Little work, however, has examined the potential role adenosine A1 receptors have in parkinsonism. Given the modulatory effects of A1 receptors on dopamine D1 receptors [2], it is possible that A1 antagonism may produce a reversal of parkinsonian symptoms, particularly those induced by D1 antagonism.

The involvement of adenosine, dopamine, and other neurotransmitter systems in parkinsonism has been my primary area of research for the last eight of my fifteen months in Dr. Salamone's behavioral pharmacology laboratory. This work has culminated in two posters at the annual Society for Neuroscience conference, as well as the preparation of a manuscript for publication. Following the completion of my undergraduate degrees in Psychology and Physiology & Neurobiology, I plan on continuing in the area of parkinsonian research in graduate school, where I intend to earn a PhD in neuroscience. This project will aid in my understanding of the complex neurochemical interactions involved in PD, as well as facilitate the development of the experimental and methodological skills essential for graduate school.

## Literature Review

Parkinsonism is a family of motor disorders, which includes idiopathic Parkinson's Disease, as well as neuroleptic-induced, pugilistic, and post-encephalitic parkinsonism. These disorders are all characterized by abnormalities in the neurochemical circuitry of the basal ganglia, particularly in reference to dopamine. These abnormalities lead to various motor impairments, including rigidity, decreased locomotion, and resting tremor [3]. However, the specific mechanisms through which specific brain circuits can lead to production of these symptoms, particularly tremor, are poorly understood.

Resting tremor is one of the hallmark symptoms of parkinsonism, yet it is one of the least understood aspects of the disorder. Tremor is defined as the involuntary oscillation of a body part, and in parkinsonism tremor generally occurs in the frequency of 3-7 Hz [3]. While this is most commonly seen in the hands, tremor in humans has also been shown to involve the jaw [3]. Tremulous jaw movements are a rodent model of this resting tremor that has been extensively validated by Salamone et al. [3, 4]. Tremulous jaw movements (TJMs) are defined as "the rapid vertical deflection of the lower jaw that resembles chewing but is not directed at any particular stimulus" [3]. They occur in the same 3-7Hz frequency that is seen with resting tremor in humans [4, 5]. Also, they can be induced by a number of pharmacological agents that produce neurochemical conditions in the basal ganglia that parallel those seen in parkinsonian individuals [5, 6]. Along this same line, TJMs can be reversed by compounds used to treat parkinsonism, such as dopamine agonists (compounds that mimic the neurotransmitter) [7], anticholinergics (compounds that block acetylcholine action) [6], and, more recently, adenosine A2A antagonists (compounds that block receptor sites) [1, 8, 9].

Adenosine is an endogenous neuromodulator that is known to play a role in the striatum, a brain structure implicated in the motor impairments seen in parkinsonism [10]. Recent research

into the neurochemistry associated with parkinsonism has focused on dopamine and adenosine interactions, particularly the relation between the D2 and A2A receptor subtypes. It is believed that these receptors are colocalized in the striatum, and that they have competing effects on the inhibitory GABA neurons in the indirect pathway [11]. Whereas D2 receptors facilitate the disinhibition of the thalamus, and subsequently the production of motor activity, A2A receptors have the opposite effect, signifying that A2A antagonism may alleviate parkinsonian symptoms [10]. Various studies have investigated this hypothesis and have found that A2A antagonists do produce a reversal of parkinsonism [1, 8, 12]. Collins et al. [13] recently found that a novel adenosine A2A antagonist reversed the motor impairment induced by chronic administration of the D2 antagonist pimozone. Conversely, it has been shown that the TJMs induced by chronic pimozone could not be reversed by the A1 antagonist DPCPX [14], suggesting that colocalization of receptors may be critical.

Extensive physiological data indicate that there is an interaction between D1 and A1 receptors in the direct pathway of the striatum [2, 10]. It is believed that decreased levels of striatal D1 activity contribute to increased thalamic inhibition, producing the motor impairments seen in parkinsonism [15]. A1 receptors in the striatum antagonistically modulate this D1 activity by changing the receptor binding characteristics of dopamine as well as inhibiting its release into the striatum [2, 10]. Due to these interactions, it is believed that antagonism of A1 receptors will reverse the parkinsonian symptoms, particularly tremor, induced by D1 antagonism. Despite what is known about DA and adenosine receptors in brain areas such as the striatum, little research has examined the implications of possible D1/A1 receptor interactions in relation to parkinsonism. The present study seeks to further elucidate this interaction and its possible relevance to novel parkinsonian treatments.

## **Experimental Design**

Three separate experiments will be used to assess the involvement of D1 and A1 receptors in the production and reversal of parkinsonian symptoms.

The first experiment will seek to elucidate the extent to which chronic administration of a D1 antagonist, SCH 39166, can produce signs of parkinsonism. Various doses will be administered to groups of male Sprague-Dawley rats for a total of seven days. On the seventh day, each rat will receive an injection thirty minutes before testing occurs. Twenty minutes later, each rat will be placed in the observation chamber to habituate for ten minutes, after which it will be observed for tremulous jaw movements. Following this, each rat will be assessed for catalepsy (an immobility response related to parkinsonism) and then placed in a stabilimeter chamber to measure locomotion (locomotor suppression also is a sign of parkinsonism). Seven days of treatment will be used because previous research has shown that these parkinsonian motor effects, especially TJMs, tend to sensitize with repeated administration [1, 3, 4].

The second experiment will examine the ability of an A1 antagonist, DPCPX, to reverse parkinsonism induced by D1 antagonism. SCH39166 (dosage determined from Experiment 1) will be administered chronically for seven days. On the seventh day, each rat will receive an injection of SCH 39166 as well as an injection of either vehicle or DPCPX thirty minutes before testing. Twenty minutes later, each rat will be placed in the observation chamber to habituate and then observed for jaw movements. Catalepsy and locomotion tests will follow. A decrease in TJMs and catalepsy, and an increase in locomotion, are expected as a result of the A1 antagonist.

To evaluate whether the effects seen in Experiment 2 are receptor subtype specific (i.e., a result of an interaction between D1 and A1 receptors specifically), the final experiment in this study will pair a D1 antagonist with the A2A antagonist MSX-3. SCH 39166 will be administered for seven days. On the seventh day, each rat will receive an injection of SCH 39166

thirty minutes before testing, followed by an injection of MSX-3 ten minutes later. After another ten minutes, the rat will be placed in the observation chamber to habituate and then observed for jaw movements. Each rat will then be assessed for catalepsy and locomotion suppression.

### **Behavioral Testing Procedures:**

The behavioral procedures to be employed in the proposed studies (TJMs, catalepsy and locomotion) have been used previously to assess motor function related to parkinsonism [e.g., 1].

**Tremulous Jaw Movements:** Each rat will be placed in a Plexiglas chamber positioned atop a wire mesh floor raised 42 cm above the table top to allow observation from all angles. Jaw movements will be counted for three 5-minute periods (15 minutes total) using a mechanical hand counter. Tremulous jaw movements are defined as vertical deflections of the lower jaw that resemble chewing but are not directed at any stimulus. Yawning, gaping, tongue protrusions, or jaw movements resulting from grooming or licking the mesh will not be counted.

**Catalepsy:** Each rat will be positioned in a catalepsy apparatus so that his forelimbs rest on the bar while his hindpaws rest on the platform. The observer will time how long it takes the rat to remove either his forelimbs from the bar (i.e., dropping down on all fours to the platform) or his hindpaws from the platform (i.e., climbing up onto the bar). Three trials will be conducted and the times measured averaged.

**Locomotion:** Each rat will be placed in a Plexiglas stabilimeter chamber inside a sound-proof casing. The floor of the chamber consists of two wire mesh panels which are attached to a central rod, allowing the deflection of four quadrants (defined as half a panel). Depression of a quadrant results in the closing of a microswitch associated with that quadrant. Each closing of a microswitch is recorded as one activity count. Locomotion sessions last 30 minutes.

Funding for this study will be provided via grants procured by Dr. Salamone. This work will be conducted according to animal protocols already approved for the Salamone laboratory.

## **Plan of Study**

The coursework for my last three semesters at the University of Connecticut is centered around an in-depth study of the anatomy and functionality of the nervous system, with particular emphasis on how this can be applied to understanding the neuronal mechanism involved in PD.

Spring 2009: For this semester the courses that I am particularly interested in are PNB 3251 and PSYC 2200. Both are concerned with the anatomical and functional aspects of the brain. PNB 3251 is more concerned with the molecular and systems level anatomy of the brain while PSYC 2200 examines the role that the brain plays in various behaviors. The two should compliment each other well and greatly aid in my understanding of the mechanisms associated with PD.

Summer 2009: I will be taking MCB 2000, which is an important course for developing an understanding of biochemical processes, which can then be applied to understanding the neurochemical aspects of PD.

Fall 2009: PNB 4400 will be an in-depth biological study of neurological disorders, which is very pertinent to my area of interest. PNB 3276 will facilitate my understanding of the complex neurochemical interactions involved in the nervous system, particularly at the cellular level. I will also be taking PNB 3263WQ, which will aid in developing my research methodology skills. Finally, I will take PSYC 5140, which is a graduate course that will further develop my understanding of neurological functionality.

Spring 2010: PSYC 3251 is concerned with more functional neuroanatomy as well as laboratory methodology. PSYC 5228 is a graduate course that concerns itself with how pharmacological agents affect the nervous system, which is particularly important for my research since the parkinsonism I will be studying is pharmacologically induced. Considering the possible relevance stem cell treatment may have in concerns to curing PD I will also take PNB 3260.

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