8-1-2011

Galantamine-induced tremulous jaw movements in rats: Reversal with adenosine A2A antagonists

Chelsea Elizabeth Leser

University of Connecticut - Storrs, steelrose97@comcast.net

Galantamine-induced tremulous jaw movements in rats: Reversal with adenosine A$_{2A}$ antagonists

The University Scholar and Honors Scholar Thesis of Chelsea E. Leser

Department of Psychology

University of Connecticut, Storrs CT 06269 USA

August 2011

Table of Contents

Acknowledgements

I would like to express my deepest and most sincere gratitude to Dr. John Salamone, my thesis advisor, for his wisdom, his guidance, and his immeasurable patience throughout the research and writing process of this thesis. Without his dedication, concern, and considerable aid, this project would not have been possible. Secondly, I would like to thank Mrs. Lyndsey Collins-Praino for her assistance and support throughout the course of this project. A special thanks is also due to the members of the Salamone Lab, especially Mr. Patrick Randall and Mr. Eric Nunes, for not only making this project a success, but also for making my experience in the lab educational, exciting and enjoyable. They helped me to gather the knowledge necessary to perform this project, and the passion and drive necessary to complete it. In addition, I would like to express my gratitude to the University of Connecticut Honors Program for making this opportunity available to me, and for providing me with the Summer Undergraduate Research Fund. Last, but certainly not least, I would like to extend my appreciation and thankfulness to my University Scholar advisors, Dr. Heather Read and Dr. Harvey Swadlow, who both offered exceptional guidance in both this project, and in my future endeavors in academia.

Abstract

Alzheimer's Disease (AD) is the most common neurodegenerative disease, affecting nearly 24.3 million people worldwide, with an estimated 4.6 million new cases every year (Ferri et al. 2005). AD is characterized primarily by a severe deficit in cognitive ability, especially in remembering past and present events. The underlying neuropathology of AD includes the presence of senile plaques and neurofibrillary tangles. In addition, patients with AD have a severe degeneration of basal forebrain cholinergic neurons, and the subsequent loss of cholinergic transmission in the cerebral cortex and other brain areas. Due to the correlation between loss of acetylcholine (ACh) and the symptoms of AD, pharmacologic treatments have aimed at increasing the amount of ACh transmission in the basal forebrain. The most common of these treatments is acetylcholinesterase (AChE) inhibitors, such as tacrine, donepezil, and galantamine. These drugs inhibit the action of AChE, which metabolizes ACh in the brain, thus resulting in an increased amount of ACh available for transmission. While these drugs produce a significant improvement in cognitive function, they also produce, as a side effect, motor impairments similar to those seen in Parkinson's disease. This effect is seen with the AChE inhibitors used to treat AD, such as galantamine, and are thought to be due to interactions between ACh and various other neurotransmitters and neuromodulators in the basal ganglia. For example, neostriatal medium spiny neurons also contain receptors for the neuromodulator, adenosine. Most notably, adenosine receptors of the A_{2A} subtype are co-localized with DA D2 receptors. Adenosine A_{2A} antagonists have been shown to reverse many of the motor side effects of DA antagonism. Due to this effect, it is reasonable to hypothesize that adenosine A_{2A} antagonists may also be beneficial in alleviating the parkinsonian side effects, most notably

resting tremor, of AChE inhibitors, such as galantamine. The present study sought to determine

if two adenosine A_{2A} antagonists, MSX-3 and MSX-4, could reverse the resting tremor produced

by administration of galantamine. Resting tremor was modeled using the extensively validated

rodent model of tremulous jaw movements. Tremulous jaw movements are defined as vertical

deflections of the lower jaw that resemble chewing but are not directed at any particular stimulus

(Salamone et al. 1998). Both MSX-3 (1.25-10 mg/kg) and MSX-4 (2.5-20 mg/kg) were capable

of reversing the tremulous jaw movements induced by galantamine (3.0 mg/kg) administration.

Due to the results observed in this study, further research must be performed to determine if

adenosine A_{2A} antagonists have a potential for treatment of idiopathic or drug-induced

parkinsonism.

Introduction

Alzheimer's Disease

     Alzheimer's Disease (AD) is a neurodegenerative disease that proceeds in an ordered, progressive fashion, passing from mild, to moderate, to very severe forms. It is estimated that nearly 24.3 million people suffer from dementia worldwide, with 4.6 million new cases every year (Ferri et al. 2005). In the U.S., approximately 4.5 million people suffer from AD, with 7% between the ages of 65 and 74 years old, 53% between the ages of 75 and 84, and 40% ages 85 and older (Hebert, et al. 2003). The most notorious symptom of AD is cognitive impairment, most commonly manifest as problems in remembering past and present events, although psychiatric symptoms and behavioral disturbances have been reported in 50-70% of patients with AD (Hamuro, et al. 2007). In the most severe cases, these symptoms include difficulty in verbal communication, total disorientation, impaired executive, visual and spatial functions, agitation, hallucinations, depression, aggressive behavior, and loss of motor control (Voisin and Vellas, 2009). The underlying neuropathology of these symptoms of AD includes various lesions or abnormal structures located in the brain surface, which typically can only be seen by postmortem examination. The most common of these neuropathological features, and also the most important for making the diagnosis of AD, are neurofibrillary tangles. These "tangles" are abnormal fibrous inclusions located in the perikaryal cytoplasm of pyramidal neurons (Perl, 2010). The second most common brain lesions associated with AD are senile (or neuritic) plaques. These plaques are very complex and varied, but most contain a core accumulation of a 4kD protein with a beta-pleated sheet configuration, and can be seen diffusely throughout the brain (Perl, 2010). While AD is a debilitating disease, there exists no known cure or highly

effective treatment, although new psychopharmacological therapies are emerging based on the involvement of the cholinergic pathways in the brain in AD.

The Cholinergic Hypothesis

As the number of patients suffering from dementia and AD continued to grow in the late 1970s, researchers began looking for the distinct patterns of dysfunction in the central nervous system that would cause the symptoms seen in dementia. While many neurotransmitter systems were studied during this time, more and more researchers began finding evidence for the involvement of acetylcholine in memory dysfunction (Bartus, et al., 1982). These early researchers found a decrease in acetylcholine (Ach) synthesis, uptake and release in the post-mortem brains of dementia and AD patients compared to the brains of patients not suffering from AD or dementia (Bowen, et al., 1976; Rylett, et al., 1983; Nilsson, et al., 1986). The findings from these studies led to the formation, and subsequently to the ongoing revision, of the cholinergic hypothesis (Contestabile, 2010). This hypothesis states that the cognitive deficits produced in AD are caused primarily by the degeneration of basal forebrain cholinergic neurons, and the subsequent loss of cholinergic transmission in the cerebral cortex and other brain areas (Bartus, et al., 1982, Francis, et al., 1999).

Acetylcholine acts as an important neuromodulator and transmitter in the central nervous system, and is intrinsically tied to the etiology of AD. Acetylcholine (ACh) and the cholinergic neurons in the brain have been linked, in many different studies, to the production of amyloid-β peptides, the principle component of the senile plaques, one of the most common brain lesions present in AD patients (Pakaski and Kalman, 2008). In general, a decrease in cholinergic function leads to an increase in the production of amyloid-β and its precursors, and vice versa

(Auld, et al., 1998, Buxbaum, et al., 1992). In addition to its influence on amyloid-β peptide in AD, the cholinergic system also exhibits a loss of the activity of choline acetyltransferase, the enzyme responsible for acetylcholine synthesis, which correlates with the cognitive dysfunctions observed clinically in AD (Davies and Maloney, 1976). Finally, AD patients experience a significant loss of the basal forebrain cholinergic neurons, and subsequently a decrease in the number of both nicotinic and muscarinic acetylcholine receptors in the cortex and hippocampus (Whitehouse, et al., 1982, Zhang, et al., 2004).

Pharmacological Treatment of Alzheimer's Disease

As stated by the cholinergic hypothesis and extensively studied by various researchers, ACh deficits in the hippocampus and cortex play a major role in the development and symptoms of AD. Due to this direct involvement, pharmacological studies have looked into ways to enhance the amount of ACh in the brain, such as the use of cholinomimetics and cholinergic receptor agonists. One of the most commonly used pharmacological treatments to enhance ACh levels has been acetylcholinesterase (AChE) inhibitors. These drugs decrease the effect of AChE, which hydrolyses ACh at the synapse, thus increasing the levels of ACh at the synapse and the subsequent effect that ACh has on the synapses (Francis, et al., 1999). Some registered and clinically used AChE inhibitors include tacrine (Cognex), donepezil (Aricept), rivastigmine (Exelon), and galanthamine (Francis, et al. 1999).

Acetylcholinesterase inhibitors were developed in essentially two generations. The first generation compound, tacrine, produced statistically significant and dose related improvements in cognitive abilities in patients with probable AD (Francis, et al., 1999). Despite these positive

results, tacrine also presents with various side effects, some life threatening, such as detrimental

effects on the hepatic and cardiovascular function, and detrimental effects of increased ACh

transmission in the peripheral nervous system, which includes nausea, vomiting, abdominal pain

and diarrhea (Francis, et al., 1999). Due to the severity of the side effects, a second generation of

AChE inhibitors needed to be developed. The second generation of AChE inhibitors, including

donepezil, rivastigmine and galanthamine, produced similar improvements in cognition as

tacrine, but without the hepatotoxicity or cardiovascular problems associated with the first

generation of inhibitors (Francis, et al. 1999). It was reported several years ago that the first

generation anticholinesterase, tacrine, could produce parkinsonian side effects including tremor

(Ott and Lannon, 1992; Cabeza-Alvarez et al. 1998). More recently, the second generation

anticholinesterases donepezil, rivastigmine and galantamine, which were developed as

alternatives that have largely replaced tacrine, also were reported to induce tremor (Arai, 2000;

Aarsland et al. 2003; Gurevich et al. 2006; McSwain et al. 2007; Litvinenko et al. 2007). In view

of these motor side effects of anticholinesterases, it is important to review the literature on the

symptoms and pathophysiology of parkinsonism.

Parkinsonism

The term, parkinsonism, refers to a family of motor disorders that are characterized by

motor dysfunctions such as bradykinesia, akinesia, postural instability, rigidity, and resting

tremor (Factor and Weiner, 1988). Bradykinesia is a slowing of movement, and is associated

with paucity of movement, reduced handwriting size, facial immobility, stooped posture, and a

shuffling gait (Bennett, et al. 1996). Akinesia is associated with a complete cessation, or

freezing, of spontaneous movement (Bennett, et al. 1996). Postural instability and rigidity as a

part of parkinsonism are defined as improper muscle tone, with no previous neurological deficits

or other markers that may point to a different pathology (Mitchell and Rockwood, 2001). The resting tremor observed in parkinsonism is an oscillatory movement of a limb or body part that is relaxed and completely supported against gravity (Crawford and Zimmerman, 2011). This tremor is often asymmetrical, occurs at rest at a frequency of 3-7 Hz, and becomes less prominent with voluntary movement (Crawford and Zimmerman, 2001; Staude, et al. 1995).

There are over 4 million people worldwide suffering from parkinsonism, with 1.5 million of those residing in the United States (Nussbaum, et al. 2003). Similar to AD, the incidence of parkinsonism increases with age; with an incidence rate of $0.8/10^5$ for ages 0-29, jumping to $25.6/10^5$ for ages 50-59, and exponentially increasing to $304.8/10^5$ for ages 80-99 (Bower, 1999). The underlying pathology for parkinsonism shows a decrease in dopaminergic function in the dorsal striatal pathway in the basal ganglia, originating in the loss of dopaminergic neurons in the substantia nigra pars compacta (Hornykiewicz, 1963). While idiopathic Parkinson's disease is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta, parkinsonism can be induced in a variety of methods that create a loss of dopamine transmission in the basal ganglia (Grealish, et al. 2010).

Anatomy of the Basal Ganglia

The basal ganglia are an integral part in the control of modulating voluntary movement, among other things. However, there are no direct connections between the basal ganglia and the spinal cord. Composed of an interconnecting network of deep brain nuclei and axon bundles, the basal ganglia are widespread throughout the brain, residing in the telencephalon, diencephalon, and midbrain (Gerfen and Wilson, 1996; Chakravarthy, et al. 2010). These seven, extensively networked, deep brain nuclei have been identified as the caudate nucleus, putamen, globus

pallidus internal (GPi) and external (GPe), subthalamic nuclei (STN), and the substantia nigra pars reticulate (SNr) and pars compacta (SNc) (Chakravarthy, et al. 2010).

The basal ganglia receive input from cortical layer 5 neurons located in the majority of the sensory-motor areas of the prefrontal cortex, including primary motor cortex and both primary and secondary somatosensory cortices, outlined in Figure 1 (Gerfen and Wilson, 1996; Chakravarthy, et al. 2010). These pyramidal neurons feed directly into the caudate, putamen, and nucleus accumbens, collectively known as the striatum (Gerfen and Wilson, 1996). Spiny projection neurons, known as medium spiny neurons, comprise of 95% of the caudate and putamen circuitry, acting as both primary input target and projection neurons of the striatum (Gerfen and Wilson, 1996). These medium spiny neurons utilize GABA (γ-amino-butyric acid) as their primary signaling neurotransmitter, therefore sending inhibitory signals to any connected nuclei when activated (Chakravarthy, et al. 2010). The other 5% of the cells within the striatum consist of interneurons that use other neurotransmitters, such as dopamine (DA), acetylcholine (ACh) and adenosine, to modulate the activity of the medium spiny neurons.

Researchers have proposed the existence of a divergent system within the basal ganglia (Graybiel, et al. 1994). In the 1994 paper, Graybiel, et al. outline a hypothesis that defines the circuitry of the basal ganglia as consisting of two major pathways, the direct and indirect, that diverge and then converge within the striatum. These pathways are differentiated by the structures to which they project, and the neurotransmitters that their interneurons utilize.

The direct pathway, also identified as the striatonigral pathway, contains neurons that express D1 receptors, in addition to substance P, dynorphin, adenosine A1, and muscarinic M4 receptors (Chakravarthy, et al. 2010; Hauber, 1998). The direct pathway neurons receive

glutamatergic input from the cortex, and dopaminergic input to D1 receptors from the SN_C.

These neurons send direct, GABAergic, axonal connections to the output structures of the basal

ganglia, the GP_i, and the SN_R (Gerfen and Surmeier, 2011). In the normally functioning basal

ganglia, the dopaminergic input from the SN_C activates the excitatory D1 receptors in the

striatum. The excitatory D1 response elicits an increase in the release of GABA from the

striatum to the GP_i and SN_R (Gerfen and Surmeier, 2011). The increased GABA transmission in

the GP_i and SN_R results in a decrease in activity of both output structures, leading to a decrease

in the release of GABA to the thalamus, and an increase in the excitatory response sent to motor

cortex (Gerfen and Surmeier, 2011). However, in a parkinsonian brain, the SN_C fails to release

the dopamine necessary for the excitatory response of the D1 receptors. This decrease in

dopaminergic transmission leads to a decreased GABA transmission in GP_i and SN_R, and

ultimately a decreased excitation of the motor cortex (Chakravarthy, et al. 2010; Gerfen and

Surmeier, 2011).

      The indirect pathway, also identified as the striatopallidal pathway, contains neurons that

express D2 receptors, in addition to enkephalin, adenosine A_{2A}, and muscarininc M1 receptors

(Chakravarthy, et al. 2010; Hauber, 1998). These neurons receive a glutamatergic input from the

cortex and a dopaminergic input from SN_C, like the direct pathway, however they do not send

projections directly to the output structures of the basal ganglia (Gerfen and Surmeier, 2011).

Neurons in the indirect pathway first project to the GP_e, which has axonal projections to the

STN, and the STN projects to the output structures of the basal ganglia (Gerfen and Surmeier,

2011). Dopaminergic input to the D2 receptors in the striatum leads to an inhibitory response,

which decreases the release of GABA by the striatum. The decreased GABA-ergic response

being sent to the GP_e leads to less inhibition of GP_e, and thus an increase in the release of GABA

by GP_e. The increased GABA transmission at the STN leads to a decrease in the release of glutamate by STN, which leads to a decreased glutamatergic response to GP_i and SN_R. The decreased glutamate transmission leads to a less excitatory response being sent to GP_i and SN_R, resulting in decreased GABA transmission to thalamus, and thus increased motor cortex activity (Gerfen and Surmeier, 2011). The indirect pathway serves as a modulatory pathway that regulates the excitatory response being sent to cortical motor areas (Chakravarthy, et al. 2010; Gerfen and Surmeier, 2011).

In a parkinsonian state, the dopaminergic input to the D2 receptors is diminished, resulting in less dopamine transmission, and thus less inhibitory action in the striatum. Less dopaminergic transmission in striatum leads to an increase in GABA release from the striatum to GP_e. The increased GABA transmission at GP_e causes an inhibition of function, leading to decreased GABA release to STN. This, in turn, leads to an increased glutamatergic transmission to GP_i and SN_R, which causes an increased GABA release onto thalamus (Gerfen and Surmeier, 2011). In the end, cortical motor areas receive less excitatory input and decreased motor output, as well as the loss of the modulatory pathway in the basal ganglia (Chakravarthy, et al. 2010; Gerfen and Surmeier, 2011).

Acetylcholine in Basal Ganglia

Acetylcholine (ACh) and cholinomimetics target and activate ionotropic nicotinic receptors and metabotropic muscarinic receptors, in both the periphery and the central nervous system. In the central nervous system, the majority of cholinergic neurons have diffuse projections to a broad area of the CNS. In the basal ganglia, especially the striatum, however, cholinergic interneurons produce very dense, localized innervations, although they only account

for approximately 2% of the striatal cell population (Zhou, et al. 2002). These interneurons provide a continuous ACh signaling of action potentials through a tonic rate of firing of approximately 5 Hz, which is only interrupted by high levels of acetylcholinesterase (AChE) to avoid desensitization of receptors (Zhou, et al. 2002).

Of all of the subtypes of both nicotinic and muscarinic receptor subtypes, M1 and M4 have been directly linked to motor functions mediated by basal ganglia (Oki, et al. 2005). The neostriatum is incredibly rich in M4 receptors, and this subtype is primarily present on the striatonigral (direct) pathway of basal ganglia (Santiago and Potter, 2001). These receptors function post-synaptically, and are found on nearly half of all medium spiny neurons in the striatum (Santiago and Potter, 2001). These M4 receptors are linked to a G_i-mediated inhibition of c-AMP production, which is in opposition to D1 receptors, which cause an increase in c-AMP production, leading to an increase in release of GABA release, as previously discussed (Gomeza, et al. 2001). Several studies have indicated that chronic administration of muscarinic agonists cause jaw movements that are described as "tremulous" and "vacuous" (Rupniak, et al. 1983; Salamone, et al. 1990). These jaw movements have been found, through video analysis, to be of the same frequency (3-7 Hz) as TJMs modeling parkinsonism, and are produced in bursts, consistent with drug-induced parkinsonism (Mayorga, et al. 1997; Salamone, et al. 1998). Pharmacological and neurochemical studies have indicated that cholinomimetic-induced jaw movements are induced by action on the muscarinic receptors in the striatum (Mayorga, et al. 1997; Salamone, et al. 1998). In addition, muscarinic antagonists, such as tacrine, have been found to be able to reverse the cholinergic-induced jaw movements, further reinforcing the idea that muscarinic ACh receptors, especially M4 receptors, play a key role in the motor functions controlled by basal ganglia (Betz, et al. 2007).

Adenosine in Basal Ganglia

Recently, studies have identified the purine nucleoside, adenosine, as being an important neuromodulator within the central nervous system, most notably within the hippocampus and basal ganglia for its role in neuronal excitability and synaptic and non-synaptic transmission (Sperlagh and Vizi, 2011). Adenosine is not a neurotransmitter in the classical sense, because it is not stored and released from vesicles (Sperlagh and Vizi, 2011). Rather, adenosine is derived from the breakdown of ATP, which is present in all metabolically active cells, leading to a large potential pool for the release of adenosine, despite the lack of vesicular storage (Dunwiddie, et al. 1997). Adenosine targets A_1, A_{2A}, A_{2B}, and A_3 receptor subtypes, all of which are G-protein coupled receptors, with A_1 and A_3 coupled to G_i, and A_{2A} and A_{2B} coupled to G_s proteins (Fredholm, et al. 2001). Adenosine has the potential to affect any area of the central nervous system, due to its origin in ATP, however, the effects of adenosine are largely controlled by receptor availability. Adenosine A_1 receptors are expressed in many brain areas, including neocortex, hippocampus, cerebellum, and brainstem. In contrast, A_{2A} receptors have a more restricted localization, with a high level of expression in the striatum and olfactory bulb, and a much lower expression level in other brain areas. Both A_{2B} and A_3 receptors have a moderate to low expression in most areas of the brain (Dixon, et al. 1996).

As previously stated, the most abundant adenosine receptor subtype in striatum is the adenosine A_{2A} receptor. These receptors are predominantly expressed on enkephalin expressing GABAergic striatopallidal (indirect pathway) neurons. On these GABAergic enkephalinergic neurons, A_{2A} receptors interact both structurally and functionally with the dopamine D2 receptors present, forming receptor heteromers and targeting common intracellular signaling cascades (Schiffmann, et al. 2007). Due to this co-localization of adenosine A_{2A} and dopamine

D2 receptors, adenosine plays an important role in the motor functions and output of the basal ganglia.

Acetylcholine/Adenosine/Dopamine Interactions within Basal Ganglia

Within the striatum, acetylcholine, DA and adenosine receptors are all localized on GABAergic medium spiny neurons (Gerfen and Surmeier, 2011). Within the striatal neurons, ACh and DA receptors both have a dense population, often within 1 micrometer of each other, resulting in a close association between the two receptors (Descarries, et al. 1997). The high concentration of ACh receptors also results in a high amount of AChE, the enzyme used to break down ACh in the synaptic cleft. The AChE plays a part in the interaction of ACh and DA by affecting the amount of ACh present in the synaptic cleft. The strong association of ACh and DA at many pre-synaptic and post-synaptic sites causes interneuronal ACh to affect DA release by acting on the nicotinic and muscarinic receptors on DA terminals in the striatum, and M1 receptors on neurons in the SN_C (Calabresi, et al. 2000). There is a long-standing hypothesis of an antagonistic balance between ACh and DA in normal striatal function (Calabresi, et al, 2000; Zhou, et al. 2002). This arose from studies that found that DA replacement treatments and anti-cholinergic treatments were both able to alleviate the motor symptoms of Parkinson's disease (Pisani, et al. 2003).

As previously stated, ACh has two different receptor subtypes, nicotinic and muscarinic. Research suggests that nicotinic receptors primarily regulate function in the ventral striatum, and play only a limited role in the dorsal striatum, which is the most vulnerable region in Parkinson's disease and the motor deficits seen in parkinsonism (Threlfell and Cragg, 2011). Recent research suggests that muscarinic ACh receptors regulate DA in a bidirectional manner, depending on the

activity of DA neurons (Threlfell, et al. 2010). Activation of muscarinic ACh receptors results in an inhibition of DA by single pulses or low frequencies of presynaptic activity, but increases DA released at high frequencies (Threlfell, et al. 2010). Thus, it raises the threshold required for DA to have an effect on striatal neurons, and has an effect similar to that of a DA depletion at lower frequencies of presynaptic activity.

Striatal medium spiny neurons also contain receptors for the neuromodulator, adenosine, with the A_{2A} receptor being the most common receptor co-localized with D2 receptors in the striatum (Sveningsson, et al. 1999). As previously mentioned, adenosine A_{2A} receptors form receptor heteromers with DA D2 receptors, interacting both structurally and functionally, and targeting common intracellular signaling cascades. Research has shown that A_{2A} and D2 receptors have an antagonistic effect on the release of GABA on striatopallidal neurons in the striatum. Thus, activating adenosine A_{2A} receptors results in an increase in GABA release, much like D1 receptors on striatonigral neurons, and activating D2 receptors results in an inhibition of GABA release (Dayne Mayfield, et al. 1996). In addition, the D2 receptor is bound to a G_i protein, which upon activation will antagonize A_{2A} function by inhibiting adenyl cyclase activation, an important step in the cascade for GABA release (Hillion, et al. 2002). Conversely, there is an intramembrane A_{2A}/D2 receptor interaction wherein stimulation of the A_{2A} receptor decreases binding of dopamine to the D2 receptor (Ferre, et al. 1991).

This antagonistic action between the two receptors has been seen in many experiments that show that adenosine A_{2A} antagonists are capable of reversing the effects of DA antagonism. In Betz, et al. 2009, administration of the adenosine A_{2A} antagonist, KW 6002 (istradefylline), was capable of reducing the motor side effects and concurrent striatal cellular activity induced by pimozide, a typical antipsychotic drug that decreases DA levels and has been shown to produce

parkinsonism. Other adenosine A_{2A} antagonists, such as MSX-3, have also been shown to

alleviate the effects of dopamine depletion in the striatum (Salamone, et al. 2008). These studies

suggest that due to the antagonistic relationship between A_{2A} and D2 receptors, adenosine A_{2A}

antagonists may be possible treatment options for Parkinson's disease and other forms of

parkinsonism.

Tremor and the Tremulous Jaw Movement Model

One of the easiest symptoms of Parkinson's disease to identify is the presence of resting

tremor, and more than 70 percent of patients with Parkinson's disease have tremor as the

presenting feature (Crawford and Zimmerman, 2011). Parkinsonian tremor in humans is often

identifiable by a characteristic "pill rolling" movement in the hands (Barbeau, 1986).

Parkinsonian tremor typically begins as a low-frequency pill rolling motion in the hands, and

then progresses to forearm protonation and supination, and elbow flexion and extension

(Crawford and Zimmerman, 2011). In addition, studies have suggested that muscle groups such

as those controlling the head, neck and jaw, can also show the resting tremor typical of

parkinsonism (Adams and Victor, 1995).

In addition to clinical observations of parkinsonian tremor, many animal studies of

parkinsonian motor symptoms have been conducted in an attempt to create a valid animal model

of parkinsonism. Over several studies, which sought to mimic the dopamine depletion in

Parkinson's disease with neuroleptic administration of dopamine antagonists that produce

parkinsonian symptoms, researchers found that rats exhibit varying orofacial movements

(Salamone, et al. 1998; Waddington 1990). These movements, in particular the spontaneous

vertical deflection of the lower jaw, were described to be analogous to the orofacial movements,

as previously mentioned, seen clinically in patients suffering from parkinsonism. Most importantly, the jaw movements were not directed at any particular stimuli or goal-oriented action, and occurred in the same 3-7 Hz frequency as tremors present in clinical Parkinson's disease. These rapid vertical deflections of the lower jaw have come to be identified as vacuous or *tremulous jaw movements* (TJMs) (Salamone, et al. 1998). The TJM model has become a valid animal model for studying the motor dysfunction in parkinsonism, and the motor effects of regulating neurochemicals. TJMs are observed in rats with DA depletion (surgically or drug-induced), neuroleptic administration, and cholinomimetic injection, among other conditions (Collins, et al. 2010; Ishiwari, et al. 2005; Salamone, et al. 1998). These conditions, which have been found to produce TJMs, are also responsible for neuroleptic-induced parkinsonism in humans.

Muscarinic agonists have been observed to produce jaw movements incredibly similar to those induced by DA depletion or antagonism (Ishiwari, et al. 2005; Salamone, et al. 1998). This affect appears to be dependent on the activation of ACh receptors in basal ganglia (Betz, et al. 2007). In addition, tacrine, an acetylcholinesterase inhibitor, has been found to produce tremulous jaw movements, and that the TJMs induced by tacrine administration are due to an increase in ventrolateral striatal ACh (Cousins, et al. 1997; 1999). Anti-parkinsonian drugs, such as levodopa, apomorphine, and other dopamine agonists, have been found to reverse the jaw movements produced by cholinomimetics and anticholinesterases (Salamone, et al. 2005). Due to the extensive experimentation on the subject, it can be reasonably concluded that there is a link between acetylcholine and parkinsonian motor symptoms. Consistent with this hypothesis, recent studies were conducted to determine if the anticholinesterase galantamine induces tremulous jaw movements (Collins et al., 2011). Systemic injections of galantamine (0.75 – 6.0

mg/kg I.P.) induced a dose-related increase in tremulous jaw movements in rats. In a second study, co-administration of the muscarinic antagonist scopolamine (0.0156-0.25 mg/kg I.P.) produced a dose dependent suppression of tremulous jaw movements induced by a 3.0 mg/kg dose of galantamine, indicating that galantamine induces these tremulous oral movements through actions on muscarinic acetylcholine receptors. In two additional experiments, analyses of freeze-frame video and electromyographic activity recorded from the lateral temporalis muscle indicated that the local frequency of these galantamine-induced jaw movements occurs in the 3-7 Hz frequency range that is characteristic of parkinsonian tremor. Based upon these studies by Collins et al., (2011), it appears that galantamine-induced tremulous jaw movements could be a useful model for studying the pharmacological modulation of experimental tremor.

As previously noted, TJMs can be induced by a DA depletion or antagonism. Due to the interactions between adenosine and dopamine in basal ganglia, studies have shown that adenosine antagonists, specifically A_{2A} antagonists, are capable of reversing the TJMs induced by dopamine depletion, such as that produced by administration of anti-psychotics (Salamone, et al. 2008). Salamone, et al. 2008 found that adenosine A_{2A} antagonism was able to reverse the tremulous jaw movements induced by the antipsychotic drugs pimozide, haloperidol, and reserpine, which all produce dopamine antagonism within basal ganglia. Due to the tremorolytic effect of adenosine A_{2A} antagonism, it can be concluded that there is a significant link between adenosine and dopamine, and that adenosine A_{2A} antagonists may be an effective treatment for the motor dysfunction produced by parkinsonism.

Present Experiments

The acetylcholinesterase inhibitor, galantamine, is frequently used for its therapeutic effects as a treatment for Alzheimer's disease. However, like other acetylcholinesterase inhibitors, galantamine is capable of producing parkinsonian side effects clinically (Grace, et al. 2009), and TJMs in preclinical research (Collins et al., 2011). Additionally, adenosine A_{2A} antagonists have been found to have a tremorolytic effect against dopamine antagonism (Salamone, et al. 2008). This study investigates the ability of adenosine A_{2A} antagonists to reverse the adverse motor side effects produced by the acetylcholinesterase inhibitor, galantamine. Two experiments were performed with two different adenosine A_{2A} antagonists, MSX-3 and MSX-4. The same dose of galantamine was used in both experiments, and was determined through previous experiments and pilot trials. The first experiment was conducted to determine if MSX-3 could significantly reduce the number of TJMs produced by galantamine, and which doses could do so maximally. The second experiment was conducted to determine if MSX-4, a novel adenosine A_{2A} antagonist, could significantly reduce the number of TJMs produced by galantamine, and which doses were the most effective.

Previous studies indicate that adenosine A_{2A} antagonists are capable of reversing the adverse motor side effects of DA antagonism (Salamone et al., 2008). In addition, DA replacement treatments are capable of alleviating the adverse motor side effects of both dopamine depletion and acetylcholinesterase inhibitors. Therefore, it is reasonable to hypothesize that, due to the complex interactions of acetylcholine, DA and adenosine in basal ganglia, adenosine A_{2A} antagonists would also be capable of reversing the TJMs induced by galantamine. It is hypothesized, in this study, that both MSX-3 and MSX-4 will significantly reduce the number of TJMs produced by galantamine.

Materials and Methods

Subjects

Adult, male Sprague-Dawley rats (n=20; Harlan Sprague Dawley, Indianapolis, IN, USA) with no previous drug experience were used for the present experiments. The rats were pair housed in a colony maintained at 23°C under a 12:12 hour light/dark cycle, with lights on at 0700 hours. The rats weighed 350-400 grams, and had ad libitum access to lab chow and water during the course of the experiment. These studies were conducted according to University of Connecticut Institutional Animal Care and Use Committee, and NIH guidelines for animal care and use.

Pharmacological Agents

Galantamine hydrobromide ((4aS, 6R, 8aS)- 5,6,9,10,11,12- hexahydro- 3-methoxy- 11-methyl- 4aH- [1]benzofuro[3a, 3, 2-ef] [2] benzazepin-6-ol), the acetylcholinesterase inhibitor used in these experiments was obtained from Tocris Bioscience (Bristol, UK). It was dissolved in 0.9% saline solution.

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, one of the adenosine A2A antagonists used in these experiments, was synthesized at the Pharmazeutisches Institut (Universität Bonn; Bonn, Germany). MSX-3 was dissolved in 0.9% saline. The pH of the MSX-3 solution was adjusted by titrating with 1.0 N NaOH until the solid drug was completely in solution. The final pH was typically 7.4 ± 0.2, although never exceeding 7.8. MSX-3 is a pro-drug that is cleaved in-vivo to the pharmacologically active adenosine A2A antagonist, MSX-2.

MSX-4 (L-valine-3-{8-[(E)-2-[3-methoxyphenyl) ethenyl]-7-methyl-1-propargylxanthine-3-yl}propyl ester hydrochloride), the other adenosine A2A antagonist used in these experiments, was synthesized at the Pharmazeutisches Institut (Universität Bonn; Bonn, Germany). MSX-4 was dissolved in de-ionized water. MSX-4 is a pro-drug of the pharmacologically active adenosine A2A antagonist, MSX-2.

Behavioral Procedures

Tremulous Jaw Movements: Rats were placed in a 30x30x30 Plexiglas chamber with a wire mesh floor, elevated 42 cm from the tabletop, that allowed for viewing from multiple angles, including underneath the chamber. 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 lower jaw was recorded using a mechanical hand counter by a trained observer, who was blind to the experimental condition of the rat. Rats were observed in three consecutive 5-min epochs, for a total of 15 min.

Dose Selection

In previous studies, experimenters have used various doses of galantamine, depending on the variable being tested. The dose of galantamine for the current study was determined based on pilot data and previous experiments (Collins, et al. 2011). The doses for MSX-3 were based on pilot data and previous experiments, and the doses for MSX-4, with the absence of previous experiments, were based on extensive pilot studies performed in the lab prior to the present study being conducted.

Experimental Procedure

Experiment 1: Effects of MSX-3 on galantamine-induced TJMs

A group of 8 rats was used to determine the effects of acute MSX-3 administration on the TJMs induced by an acute dose of galantamine. Subjects were all administered an acute dose of 3 mg/kg of galantamine via IP injection, with a lead time of 20 minutes, determined through previous pilot studies. Rats were also administered the following doses of MSX-3 via IP injection: 0.9% saline vehicle, 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg, and 10.0 mg/kg. Doses were administered in a within-groups design, with a lead time of 10 minutes, determined through previous pilot studies. All rats received every drug treatment in a randomly varied order (one treatment per week). Rats were assessed for TJMs by an experimentally blind, trained observer manually counting the number of TJMs with a hand counter for three 5-minute epochs. The experiment was conducted over a period of 5 weeks.

Experiment 2: Effects of MSX-4 on galantamine-induced TJMs

A group of 12 rats was used to determine the effects of acute MSX-4 administration on the TJMs induced by an acute dose of galantamine. Subjects were all administered an acute dose of 3 mg/kg of galantamine via IP injection, with a lead time of 20 minutes, determined through previous pilot studies. Rats were also administered the following doses of MSX-4 via IP injection: 0.9% saline vehicle, 2.5 mg/kg, 5.0 mg/kg, 10.0 mg/kg, and 20.0 mg/kg. Doses were administered in a within-groups design, with a lead time of 10 minutes, determined through previous pilot studies. All rats received every drug treatment in a randomly varied order (one treatment per week). Rats were assessed for TJMs by an experimentally blind, trained observer manually counting the number of TJMs with a hand counter for three 5-minute epochs. The experiment was conducted over a period of 5 weeks.

Data Analysis

      The behavioral data for all experiments were analyzed using a between-groups analysis of variance (ANOVA). Average tremulous jaw movements over the three five-min observation epochs were calculated and then used in the ANOVA calculations. A computerized statistical program (SPSS 10.1 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 total number of comparisons was restricted to the number of treatments minus one (Keppel, 1991).

Results

Experiment 1: The effects of the adenosine A_{2A} antagonist, MSX-3, on galantamine-induced tremulous jaw movements

Figure 2 shows that the co-administration of MSX-3 with 3 mg/kg galantamine produces a dose-dependent decrease in the number of TJMs induced by galantamine. Repeated measures ANOVA revealed that there was a significant overall effect of MSX-3 on the amount of tremulous jaw movements induced by galantamine at 3 mg/kg ($F(4,28) = 5.364$; $p < 0.01$). Planned comparisons showed that the 5.0 and 10.0 mg/kg doses of MSX-3 significantly reduced the tremulous jaw movements induced by 3.0 mg/kg galantamine (i.e., compared to galantamine plus saline; $p < 0.01$).

Experiment 2: The effects of the adenosine A_{2A} antagonist, MSX-4, on galantamine-induced tremulous jaw movements

Figure 3 shows that the co-administration of MSX-4 with 3 mg/kg galantamine produces, like MSX-3, a dose dependent decrease in the number of TJMs induced by galantamine. Repeated measures ANOVA revealed that there was a significant overall effect of MSX-4 on the amount of tremulous jaw movements induced by galantamine at 3 mg/kg ($F(4,44) = 3.38$; $p < 0.05$). Planned comparisons showed that the 5.0, 10.0, and 20.0 mg/kg doses of MSX-4 significantly reduced the tremulous jaw movements induced by 3.0 mg/kg galantamine (i.e., compared to galantamine plus saline; $p < 0.01$).

Discussion

As described above, it has recently been demonstrated that the anticholinesterase galantamine could induce a very robust TJM response in rats (Collins et al., 2011). The level of TJM activity observed in the present experiments after administration of 3.0 mg/kg galantamine plus vehicle (i.e., mean TJMs of 70-80 movements per 5 min period) were comparable to those reported in the dose/response experiment described in Collins et al., (2011). The present studies investigated the ability of two adenosine A_{2A} antagonists, MSX-3 and MSX-4, to reverse the tremulous jaw movements (TJMs) induced by the acetylcholinesterase inhibitor, galantamine. Co-administration of MSX-3 with galantamine resulted in a dose-dependent reduction of TJMs, with significant reduction occurring at doses 5.0 mg/kg and 10.0 mg/kg (Figure 2). Additionally, co-administration of MSX-4 with galantamine also resulted in a dose-dependent reduction of TJMs, with significant reversal occurring at doses 5.0 mg/kg, 10.0 mg/kg, and 20.0 mg/kg (Figure 3). These results are supported by previous work with adenosine A_{2A} antagonists that also show suppression of parkinsonian tremor (Betz, et al. 2009; Correa, et al. 2004; Ishiwari, et al. 2007; Salamone, et al. 2008). Based on these findings, it seems that the interaction of acetylcholine and dopamine in basal ganglia plays a significant role in the motor dysfunctions present in parkinsonism, and that the interactions of adenosine with DA are capable of having a therapeutic effect on those motor dysfunctions.

Adenosine A_{2A} antagonists have been found to have a tremorolytic effect when co-administered with an acetylcholinesterase inhibitor such as galantamine. Previously, the tremorolytic effects of adenosine A_{2A} antagonists, such as MSX-3, SCH58261, and KW6002, had primarily been tested with DA antagonists, which block DA receptors, and muscarinic

agonists, which increase acetylcholine levels in the brain. In both DA antagonism and muscarinic agonism, adenosine A_{2A} antagonists are capable of reversing the tremulous jaw movements produced (Betz, et al. 2009; Collins, et al. 2010; Salamone, et al. 2008). The present study supported the evidence that adenosine A_{2A} antagonists have a tremorolytic effect in parkinsonism. Also, the tremorolytic effect of the adenosine A_{2A} antagonists, MSX-3 (figure 2) and MSX-4 (figure 3) was found to be dose dependent, which is consistent with, and supported by, previous research (Collins, et al. 2010; Betz, et al. 2009). Similar findings have been reported using the adenosine A_{2A} antagonists SCH58251, ST1535 and ANR94 to reduce the tremulous jaw movements induced by a low dose (2.5 mg/kg) of the acetylcholinesterase inhibitor, tacrine (Pinna, et al. 2010; Simola, et al. 2004; Tronci, et al. 2007). Together with these previous findings, the present results with the tremulous jaw movement model suggest that the adenosine A_{2A} antagonists MSX-3 and MSX-4 could be useful for attenuating the motor side effects of acetylcholinesterase inhibitors seen in humans.

The adenosine A_{2A} antagonists, MSX-3 and MSX-4, are both pro-drugs of the pharmacologically active adenosine A_{2A} antagonist MSX-2, and are both cleaved to MSX-2 in vivo as their mechanism of action (Vollman, et al. 2008). The drug, MSX-3, has been extensively studied for its tremorolytic effects, co-administered with a variety of other drugs (Collins, et al. 2010; Ishiwari, et al. 2007; Salamone, et al. 2008). The drug, MSX-4, however, is a recently synthesized, novel adenosine A_{2A} antagonist that has not previously been characterized for its behavioral effects. While MSX-3 is effective at reversing motor dysfunctions, it is a difficult drug to make, that has to remain at an ideal pH for full efficacy, and has no potential of being an orally administered drug (Vollman, et al. 2008). The novel adenosine A_{2A} antagonist, MSX-4, is much easier to get into solution, needing only to be dissolved in distilled water, and

has the potential to be an orally administered drug (Vollman, et al. 2008). This potential for oral administration is crucial for a drug to be successful clinically, as it is easier for clinical patients to take a drug orally than it is to obtain a therapeutic effect through repeated injections of a drug. Due to its potential for oral administration and efficacy in reversing the tremulous jaw movements induced by the acetylcholinesterase inhibitor, galantamine, MSX-4 is an especially good candidate for clinical trials aimed at reducing both idiopathic and drug-induced parkinsonism, and further research is necessary.

References

Aarsland, D., Hutchinson, M., Larsen, J.P. Cognitive, psychiatric, and motor response to galantamine in Parkinson's disease with dementia. *International Journal of Geriatric Psychiatry* (2003). 18, 937- 941.

Adams, R. D. and Victor, M. (1995) Tremors, myoclonus, spasms and tics. In: Principles of Neurology, pp. 69–77. McGraw–Hill, New York.

Arai, M. Parkinsonism onset in a patient currently using tiapride and donepzil. *Internal Medicine* (2000), 39, 863.

Auld, D.S., Kar, S., Quirion, R. Beta-amyloid peptides as direct cholinergic neuromodulators: a missing link?. *Trends in Neuroscience* (1998). vol. 21, pp. 43-49.

Barbeau, A. (1986) Parkinson's disease: clinical features and etiopathology. In: Handbook of Clinical Neurology, vol. 5 (49): Extrapyramidal Disorders, pp. 87–152. Eds Vinken, P. J., Bruyn, G. N. and Klawans, H. L. Elsevier, North Holland.

Bartus, R.T., Dean, R.L. III, Beer, B., Lippa, A.S. The cholinergic hypothesis of geriatric memory dysfunction. *Science* (1982). vol. 217(4558), pp. 408-414.

Bennett, D.A., Beckett, L.A., Murray, A.M., Shannon, K.M., Goetz, C.G., Pilgrim, D.M., Evans, D.A. Prevalence of parkinsonian signs and associated mortality in a community population of older people. *The New England Journal of Medicine* (1996). vol. 334, pp. 71-76.

Betz, A.J., McLaughlin, P.J., Burgos, M., Weber, S.M., Salamone, J.D. The muscarinic receptor antagonist tropicamide suppresses tremulous jaw movements in a rodent model of

parkinsonian tremor: possible role of M4 receptors. *Psychopharmacology* (2007). vol. 194, pp. 347-359.

Betz, A.J., Vontell, R., Valenta, J., Worden, L., Sink, K.S., Font, L., Correa, M., Sager, T.N., Salamone, J.D. Effects of the adenosine A_{2A} antagonist KW 6002 (istradefylline) on pimozide-induced oral tremor and striatal c-Fos expression: comparisons with the muscarinic antagonist tropicamide. *Neuroscience* (2009). vol. 163(1), pp. 97-108.

Bourke, D., Druckenbrod, R.W. Possible association between donepezil and worsening Parkinson's disease. *Annals of Pharmacotherapy*, (1998). 32, 610-611.

Bowen, D.M., Smith, C.B., White, P., Davison, A.N., Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies. *Brain* (1976). vol. 99(3), pp. 459-496.

Bower, J.H., Maraganore, D.M., McDonnell, S.K., and Rocca, W.A. Incidence and Distribution of Parkinsonism in Olmsted County, Minnesota, 1976-1990. *Neurology*, (1999) vol. 52, pp. 1214-1220.

Buxbaum, J.D., Oishi, M., Chen, H.I., Pinkas-Kramarski, R., Jaffe, E.A., Gandy, S.E., Greengard, P. Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor. *Proceedings of the National Academy of Science of the United States of America* (1992). vol. 89(2), pp. 10075-10078.

Cabeza-Alvarez, C.I., Gonzalez-Rubio, M., Garcia-Montero, R., Alvarez-Tejerina, A. Parkinsonian syndrome related to tacrine. *Neurologia* 1999; 14(2): 96.

Calabresi, P., Centonze, D., Gubellini, P., Pisani, A., Bernardi, G. Acetylcholine-mediated modulation of striatal function. *Trends in Neuroscience* (2000). vol. 23(3), pp. 120-126.

Chakravarthy, V.S., Joseph, D., Bapi, R.S. What do the basal ganglia do? A modeling

    perspective. *Biological Cybernetics* (2010). vol. 103(3), pp. 237-253.

Collins, L.E., Galtieri, D.J., Brennum, L.T., Sager, T.N., Hockemeyer, J., Muller, C.E., Hinman,

    J.R., Chrobak, J.J., Salamone, J.D. Oral tremor induced by the muscarinic agonist

    pilocarpine is suppressed by the adenosine A_{2A} antagonists MSX-3 and SCH58261, but

    not the adenosine A1 antagonist DPCPX. *Pharmacology, Biochemistry and Behavior*

    (2010). vol. 94(4), pp. 561-569.

Collins, L. E., Galtieri, D. J., Collins, P., Jones, S. K., Port, R. G., Paul, N. E., & ... Salamone, J.

    D. (2010). Interactions between adenosine and dopamine receptor antagonists with

    different selectivity profiles: Effects on locomotor activity. *Behavioural Brain Research*,

    211(2), 148-155.

Collins, L.E., Paul, N.E., Abbas, S.F., Leser, C.E., Podurgiel, S.J., Galtieri, D.J., Chrobak, J.J.,

    Baqi, Y., Muller, C.E., Salamone, J.D. Oral tremor induced by galantamine in rats: A

    model of the parkinsonian side effects of cholinomimetics used to treat Alzheimer's

    disease. *Pharmacology, Biochemistry, and Behavior* (2011). vol. 99(3), pp. 414-422.

Contestabile, A. The history of the cholinergic hypothesis. *Behavioral Brain Research* (2010).

Correa, M., Wisniecki, A., Betz, A., Dobson, D.R., O'Neill, M.J., Salamone, J.D. The adenosine

    A_{2A} antagonist KF17837 reverses the locomotor suppression and tremulous jaw

    movements induced by haloperidol in rats: possible relevance to parkinsonism.

    Behavioural Brain Research (2004). vol. 481(1-2), pp. 47-54.

Cousins, M.S., Carriero, D.L., Salamone, J.D. Tremulous jaw movements induced by the

    acetylcholinesterase inhibitor tacrine: effects of antiparkinsonian drugs. *European*

    Journal of Pharmacology (1997). vol. 322(2-3), pp. 137-145.

Cousins, M.S., Finn, M., Trevitt, J., Carriero, D.L., Conlan, A., Salamone, J.D. The role of ventrolateral striatal acetylcholine in the production of tacrine-induced jaw movements. *Pharmacology, Biochemistry, and Behavior* (1999). vol. 62(3), pp. 439-447.

Crawford, P., Zimmerman, EE. Differentiation and diagnosis of tremor. *American Family Physician* (2011). vol. 83(6), pp. 697-702.

Davies, P., Maloney, A.J. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* (1976). vol. 2, pp. 1403.

Dayne Mayfield, R., Larson, G., Orona, R.A., Zahniser, N.R. Opposing actions of adenosine A_{2A} and dopamine D2 receptor activation on GABA release in the basal ganglia: evidence for an A_{2A}/D2 receptor interaction in globus pallidus. *Synapse* (1996). vol. 22(2), pp. 132-138.

Descarries, L., Gisiger, V., Steriade, M. Diffuse transmission by acetylcholine in the CNS. *Progress in Neurobiology* (1997). vol. 53(5), pp. 603-625.

Dixon, A. K., Gubitz, A. K., Sirinathsinghji, D. J., Richardson, P.J., Freeman, T. C. Tissue distribution of adenosine receptor mRNAs in the rat. *Br. J. Pharmacol.* (1996). vol. 118, pp. 1461- 1468.

Dunwiddie, T. V.; Diao, L.; Proctor, W. R. Adenine nucleotides undergo rapid, quantitative conversion to adenosine in the extracellular space in rat hippocampus. *Journal of Neuroscience* (1997). vol. 17, pp. 7673-7682.

Factor, S. A. and Weiner, W. J. (1988) The current clinical picture of Parkinson's disease. In: Neurology, pp. 1–9. Eds F. Hefty and W. J. Weiner. Plenum Press, New York.

Ferre, S., von Euler, G., Johansson, B., Fredholm, B.B., Fuxe, K. Stimulation of high-affinity adenosine A_2 receptors decreases the affinity for dopamine D2 receptors in rat striatal

membranes. *Proceedings of the National Academy of Sciences of the United States of America* (1991). vol. 88(16), pp. 7238-7241.

Ferri, C., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., et al. Global prevalence of dementia: a Delphi consensus study. *The Lancet* (2005). vol. 366(9503), pp. 2112-2117.

Francis, P.T., Palmer, A.M., Snape, M., Wilcock, G.K. The cholinergic hypothesis of Alzheimer's disease: a review of progress. *Journal of Neurology, Neurosurgery and Psychiatry* (1999). vol. 66, pp. 137-147.

Fredholm, B. B., AP, I. J., Jacobson, K. A., Klotz, K. N., Linden, J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacological Reviews* (2001). vol. 53, pp. 527-552.

Gerfen CR, Wilson CJ (1996) The basal ganglia. In: Swanson LW, Bjorklund A, Hokfelt T (eds) Handbook of chemical neuroanatomy. Elsevier, Amsterdam, pp 371–468

Gerfen, C.R., Surmeier, D.J. Modulation of striatal projection systems by dopamine. *Annual Review of Neuroscience* (2011). vol. 34, pp. 441-466.

Gomeza, J., Zhang, L., Kostenis, E., Felder, C.C., Bymaster, F.P., Brodkin, J., Shannon, H., Xia, B., Duttaroy, A., Deng, C.X., Wess, J. Generation and pharmacological analysis of M2 and M4 muscarinic receptor knockout mice. *Life Sciences* (2001). vol. 68(22-23), pp. 2457-2466.

Grace, J., Amick, M.M., Friedman, J.H. A double blind comparison of galantamine hydrobromide ER and placebo in Parkinson disease. *Journal of Neurology, Neurosurgery and Psychiatry* (2009). vol. 80(1), pp. 18-23.

Gurevich, T.Y., Shabtai, H., Korczyn, A.D., Simon, E.S., Giladi, N. Effect of rivastigmine on tremor in patients with Parkinson's disease and dementia. *Movement Disorders* (2006), 21, 1663-1666.

Graybiel, A. M., Aosaki, T., Flaherty, A. W. & Kimura, M. The basal ganglia and adaptive motor control. *Science* (1994) vol. 265, pp. 1826–1831.

Grealish, S., Mattsson, B., Draxler, P., Bjorklund, A. Characterisation of behavioural and neurodegenerative changes induced by intranigral 6-hydroxydopamine lesions in a mouse model of Parkinson's disease. *European Journal of Neuroscience* (2010). vol. 31(12), pp. 2266-2278.

Hamuro, A., Isono, H., Sugai, Y., Torii, S., Furuta, N., Mimura, M., Kamijima, K. Behavioral and psychological symptoms of dementia in untreated Alzheimer's disease patients. *Psychogeriatrics* (2007). vol. 7, pp. 4-7.

Hauber, W. Involvement of basal ganglia transmitter systems in movement initiation. *Progress in Neurobiology* (1998). vol. 56(5), pp. 507-540.

Hebert, L., Scherr, P., Bienias, J., Bennett, D., & Evans, D. Alzheimer Disease in the US Population. *Archives of Neurology* (2003). vol. 60(8), pp. 1119-1122.

Hillion, J., Canals, M., Torvinen, M., Casado, V., Scott, R., Terasmaa, A., Hansson, A., Watson, S.,Olah, M.E., Mallol, J., Canela, E.I., Zoli, M., Agnati, L.F., Ibanez, C.F., Lluis, C., Franco, R., Ferre, S., Fuxe, K. Coaggregation, cointernalization, and codesensitization of adenosine A_{2A} receptors and dopamine D2 receptors. *Journal of Biological Chemistry* (2002). vol. 277(20), pp. 18091-18097.

Hornykiewicz, O. The tropical localization and content of noradrenalin and dopamine (3-hydroxytyramine) in the substantia nigra of normal persons and patient with Parkinson's disease. *Wiener klinische Wochenschrift* (1963). vol. 75, pp. 309-312.

Ishiwari, K., Betz, A., Weber, S., Felsted, J., Salamone, J.D. Validation of the tremulous jaw movement model for assessment of the motor effects of typical and atypical antipsychotices: effects of pimozide (Orap) in rats. *Pharmacology, Biochemistry, and Behavior* (2005). vol. 80(2), pp. 351-362.

Ishiwari, K., Madson, L.J., Farrar, A.M., Mingote, S.M., Valenta, J.P., DiGianvittorio, M.D., Frank, L.E., Correa, M., Hockemeyer, J., Muller, C., Salamone, J.D. Injections of the selective adenosine A_{2A} antagonist MSX-3 into the nucleus accumbens core attenuates the locomotor suppression induced by haloperidol in rats. *Behavioural Brain Research* (2007). vol. 178(2), pp. 190-199.

Keppel, 1991 G. Keppel, Design and analysis: a researcher's handbook, Prentice Hall (1991).

Litvinenko, I.V., Odinak, M.M., Mogil'naya, V.I., Emelin, A.Y. Efficacy and safety of galantamine (reminyl) for demenia in patients with Parkinson's disease (an open controlled trial). *Neuroscience and Behavioral Physiology* (2008). 38, 937-945.

Maidment, I.D., Fox, C., Boustani, M. A review of studies describing the use of acetyl cholinesterase inhibitors in Parkinson's disease dementia. *Acta Psychiatrica Scandinavica* (2005). vol. 111, pp. 430-409.

Mayorga A.J., Carriero D.L., Cousins M.S., Gianutsos G., Salamone J.D.. Tremulous jaw movements produced by acute tacrine administration: Possible relation to parkinsonian side effects. *Pharmacology, Biochemistry and Behavior* (1997). vol. 56, pp. 273–279.

McSwain, M.L., Forman, L.M. Severe parkinsonian symptom development on combination
treatment with tacrine and haloperidol. *Journal of Clinical Psychopharmacology*, (1995).
15, 284.

Mitchell, S.L., Rockwood, K. Defining parkinsonism in the Canadian Study of Health and
Aging. *International Psychogeriatrics* (2001). vol. 13, pp. 107-113.

Nilsson, L., Nordberg, A., Hardy, J., Wester, P., Winblad, B. Physostigmine restores 3H-
acetylcholine efflux from Alzheimer brain slices to normal level. *Journal of Neural
Transmission* (1986). vol. 67(3-4), pp. 275-285.

Nussbaum RL & Ellis CE. Alzheimer's disease and Parkinsons's disease. *New England Journal
of Medicine* (2003). vol. 348 (14), pp. 1356-1364.

Oki, T., Takagi, Y., Inagaki, S., Taketo M.M., Manabe, T., Matsui, M., Yamada, S. Quantitative
analysis of binding parameters of [3H]N-methylscopolamine in central nervous system of
muscarinic acetylcholine receptor knockout mice. *Brain Research: Molecular Brain
Research* (2005). vol. 133(1), pp. 6-11.

Ott, B.R. & Lannon, M.C. Exacerbation of parkinsonism by tacrine. *Cinical
Neuropharmacology*, (1992). 15, 322-325.

Pakaski, M., Kalman, J. Interactions between the amyloid and cholinergic mechanisms in
Alzheimer's disease. *Neruochemistry International* (2008). vol. 53(5), pp. 103-111.

Perl, Daniel. Neuropathology of Alzheimer's Disease. *Mount Sinai Journal of Medicine* (2010).
vol. 77(1).

Pinna A, Schintu N, Simola N, Volpini R, Pontis S, Cristalli G, Morelli M. A new ethyladenine
antagonist of adenosine A(2A) receptors: Behavioral and biochemical characterization as
an antiparkinsonian drug. *Neuropharmacology* (2010). vol. 58(3), pp. 613-623.

Pisani, A., Bonsi, P., Centonze, D., Gubellini, P., Bernardi, G., Calabresi, P. Targeting striatal cholinergic interneurons in Parkinson's disease: focus on metabotropic glutamate receptors. *Neuropharmacology* (2003). vol. 45(1), pp. 45-56.

Rupniak NMJ, Jenner P, Marsden CD. Cholinergic modulation of perioral behavior induced by chronic neuroleptic administration to rats. *Psychopharmacology* (1983). vol. 79, pp. 226–230.

Rylett, R.J., Ball, M.J., Colhuon, E.J. Evidence for high affinity choline transport in synaptosomes prepared from hippocampus and neocortex of patients with Alzheimer's disease. *Brain Research* (1983). vol 289, pp. 169-175.

Salamone, J.D., Carlson, B.B., Rios, C., Lentini, E., Correa, M., Wisniecki, A., Betz, A. Dopamine agonists suppress cholinomimetic-induced tremulous jaw movements in an animal model of Parkinsonism: tremorolytic effects of pergolide, ropinirole and CY 208-243. *Behavioural Brain Research* (2005). vol. 156(2), pp. 173-179.

Salamone, J.D., Betz, A.J., Ishiwari, K., Felsted, J., Madson, L., Mirante, B., Clark, K., Font, L., Korbey, S., Sager, T.N., Hockemeyer, J., Muller, C.E. Tremorolytic effects of adenosine A_{2A} antagonists: implications for parkinsonism. *Frontiers in Bioscience* (2008). vol. 13, pp. 3594-3605.

Salamone, J.D., Ishiwari, K., Betz, A.J., Farrar, A.M., Mingote, S.M., Font, L., Hockemeyer, J., Muller, C., Correa, M. Dopamine/adenosine interactions related to locomotion and tremor in animal models: Possible relevance to parkinsonism. *Parkinsonism and Related Disorders* (2008). vol. 14, pp. 130-134.

Salamone J.D., Johnson C.J., McCullough L.D., Steinpreis R.E. Lateral striatal cholinergic mechanisms involved in oral motor activities in the rat. *Psychopharmacology* (1990). vol. 102, pp. 529–534.

Salamone, J.D., Mayorga, A.J., Trevitt, J.T., Cousins, M.S., Conlan, A., Nawab, A. Tremulous jaw movements in rats: a model of parkinsonian tremor. *Progress in Neurobiology* (1998). vol. 56(6), pp. 591-611.

Santiago, M.P., Potter, L.T. Biotinylated m4-toxin demonstrates more M4 muscarinic receptor protein on direct than indirect striatal projection neurons. *Brain Research* (2001). vol. 894(1), pp. 12-20.

Schiffmann, S.N., Fisone, G., Moresco, R., Cunha, R.A., Ferre, S. Adenosine A_{2A} receptors and basal ganglia physiology. *Progress in Neurobiology* (2007). vol. 83(5), pp. 277-292.

Simola, N., Fenu, S., Baraldi, P.G., Tabrizi, M.A., Morelli, M. Blockade of adenosine A2A receptors antagonizes parkinsonian tremor in the rat tacrine model by an action on specific striatal regions. *Experimental Neurology* (2004). vol. 189, pp. 182-188

Sperlagh, B., Vizi, E.S. The role of extracellular adenosine in chemical neurotransmission in the hippocampus and basal ganglia: Pharmacological and clinical aspects. *Current Topics in Medicinal Chemistry* (2011). vol. 11, pp. 1034-1046.

Staude, G., Wolf, W., Ott, M., Oertel, W. H., Dengler, R. Tremor as a factor in prolonged reaction times of parkinsonian patients. *Movement Disorders*. (1995) vol. 10, pp. 153–162.

Svenningsson P., Le Moine C., Fisone G., Fredholm B.B. (1999). Distribution, biochemistry and function of striatal adenosine A2A receptors. *Progress in Neurobiology, 59*(4), 355-96.

Threlfell, S., Clements, M.A., Khodai, T., Pienaar, I.S., Exley, R., Wess, J., Cragg, S.J. Striatal

muscarinic receptors promote activity dependence of dopamine transmission via distinct

receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. *The*

Journal of Neuroscience (2010). vol. 30(9), pp. 3398-3408.

Threlfell, S., Cragg, S.J. Dopamine signaling in dorsal versus ventral striatum: the dynamic role

of cholinergic interneurons. *Frontiers in Systems Neuroscience* (2011). vol. 5(11).

Tronci, E., Simola, N., Borsini, F., Schintu, N., Frau, L., Carminati, P., Morelli, M.

Characterization of the antiparkinsonian effects of the new adenosine A2A receptor

antagonist ST1535: Acute and subchronic studies in rats. *European Journal of*

Pharmacology (2007). vol. 566, pp. 94-102.

Voisin, T., Vellas, B. Diagnosis and treatment of patients with severe Alzheimer's disease.

Drugs and Aging (2009). vol. 26(2), pp. 135-144.

Vollman, K., Qurishi, R., Hockemyer, J., Muller, C. Synthesis and properties of a new water-

soluble prodrug of the adenosine A2A receptor antagonist MSX-2. *Molecules* (2008). vol.

13, pp. 348-359.

Waddington, 1990 J.L. Waddington, Spontaneous orofacial movements induced in rodents by

very long-term neuroleptic drug administration: phenomenology, pathophysiology and

putative relationship to tardive dyskinesia, *Psychopharmacology* (1990). vol. 101, pp.

431–447.

Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T., Delon, M.R. Alzheimer's

and senile dementia: loss of neurons in the basal forebrain. *Science* (1982). vol. 215, pp.

1237-1239.

Zhang, L., Zhou, F.M., Dani, J.A. Cholinergic drugs for Alzheimer's disease enhance in vitro

       dopamine release. *Molecular Pharmacology* (2004). vol. 66, pp. 538-544.

Zhou, F.M., Wilson, C.J., Dani, J.A. Cholinergic interneuron characteristics and nicotinic

       properties in the striatum. *Journal of Neurobiology* (2002). vol. 53(4). pp. 590-605.

Figure Captions

Figure 1: Functional Anatomy of the Basal Ganglia:

Derived from Chakravarthy, et al. 2010; shows in simplified form the different functional

connections between the nuclei of the basal ganglia. Also shown are the primary

neurotransmitters used in the connection between two nuclei.

Figure 2: Effects of the adenosine A_{2A} antagonist, MSX-,3 on galantamine-induced tremulous

jaw movements:

Figure shows mean ($\pm$ SEM) TJMs by rats during a 5 minute session. The adenosine A_{2A}

antagonist MSX-3 is capable of significantly reducing the number of TJMs induced by

the acetylcholinesterase inhibitor galantamine at higher doses.

(*: $p < .05$; ***: $p < .001$)

Figure 3: Effects of the adenosine A_{2A} antagonist, MSX-4, on galantamine-induced tremulous

jaw movements:

Figure shows mean ($\pm$SEM) TJMs by rats during a 5 minute session. The adenosine A_{2A}

antagonist MSX-4 is capable of significantly reducing the number of TJMs induced by

the acetylcholinesterase inhibitor galantamine.

(*: $p < .05$; **: $p < .01$)
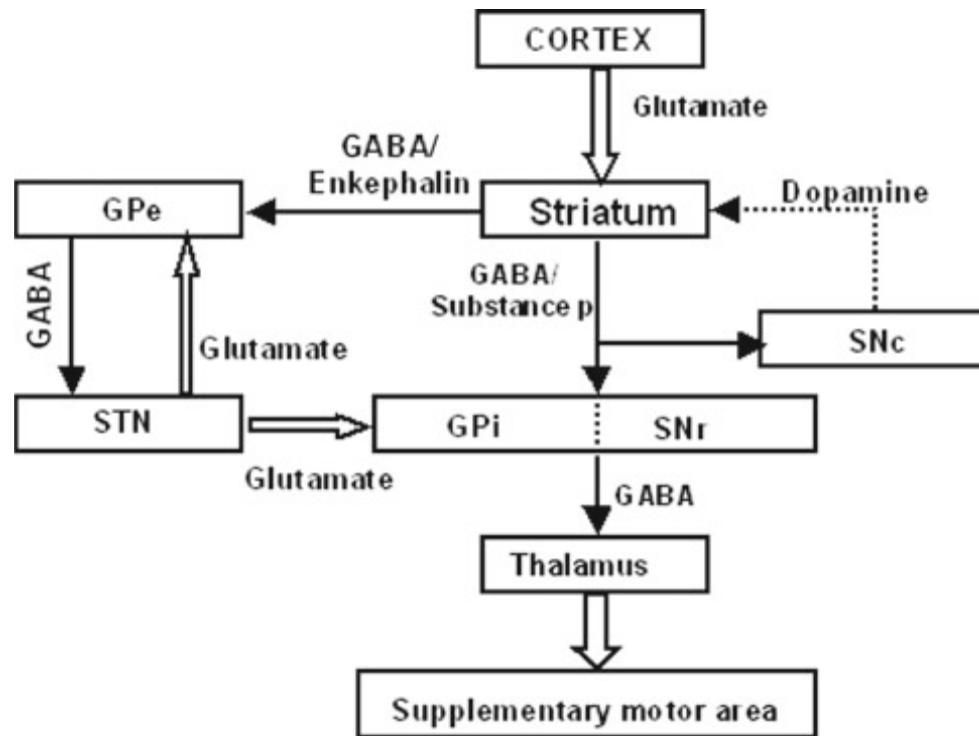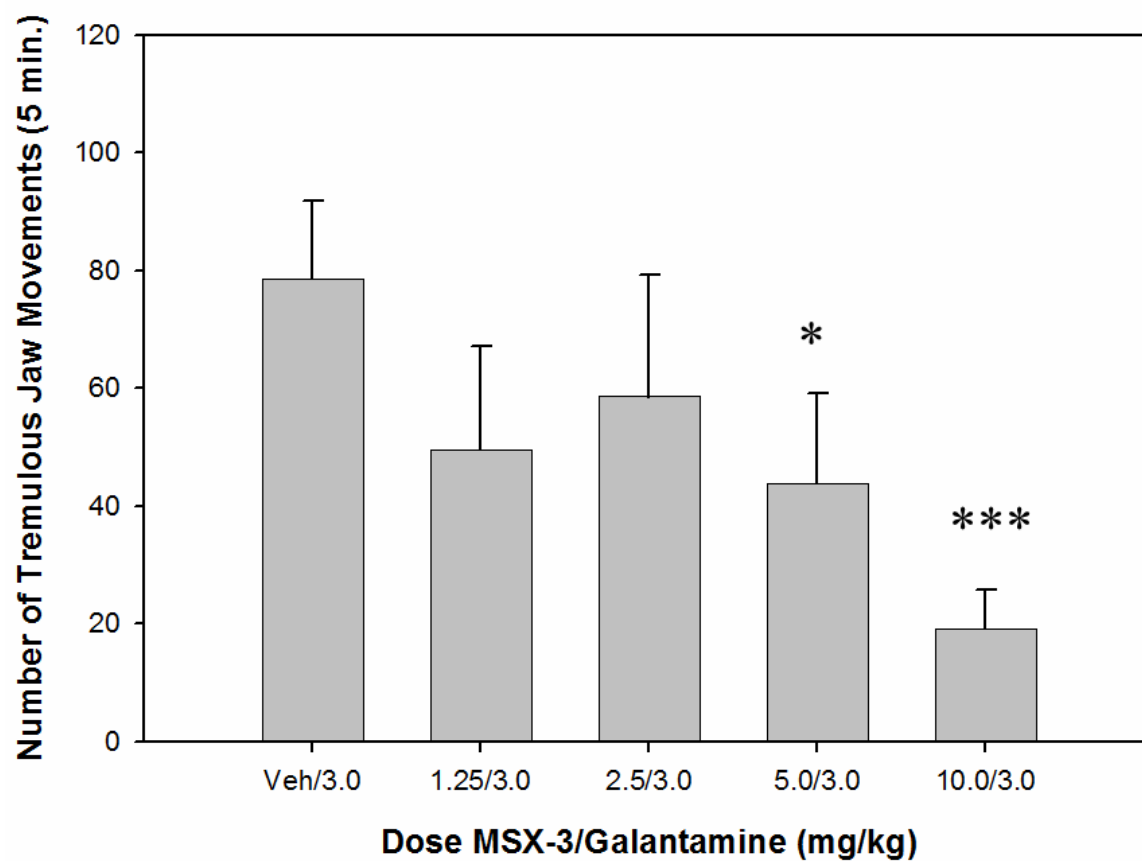
Functional Anatomy of the Basal Ganglia

Figure 1

Effects of the adenosine A$_{2A}$ antagonist, MSX-3, on galantamine-induced tremulous jaw movements

Figure 2

Effects of the adenosine A$_{2A}$ antagonist, MSX-4, on galantamine-induced tremulous jaw movements
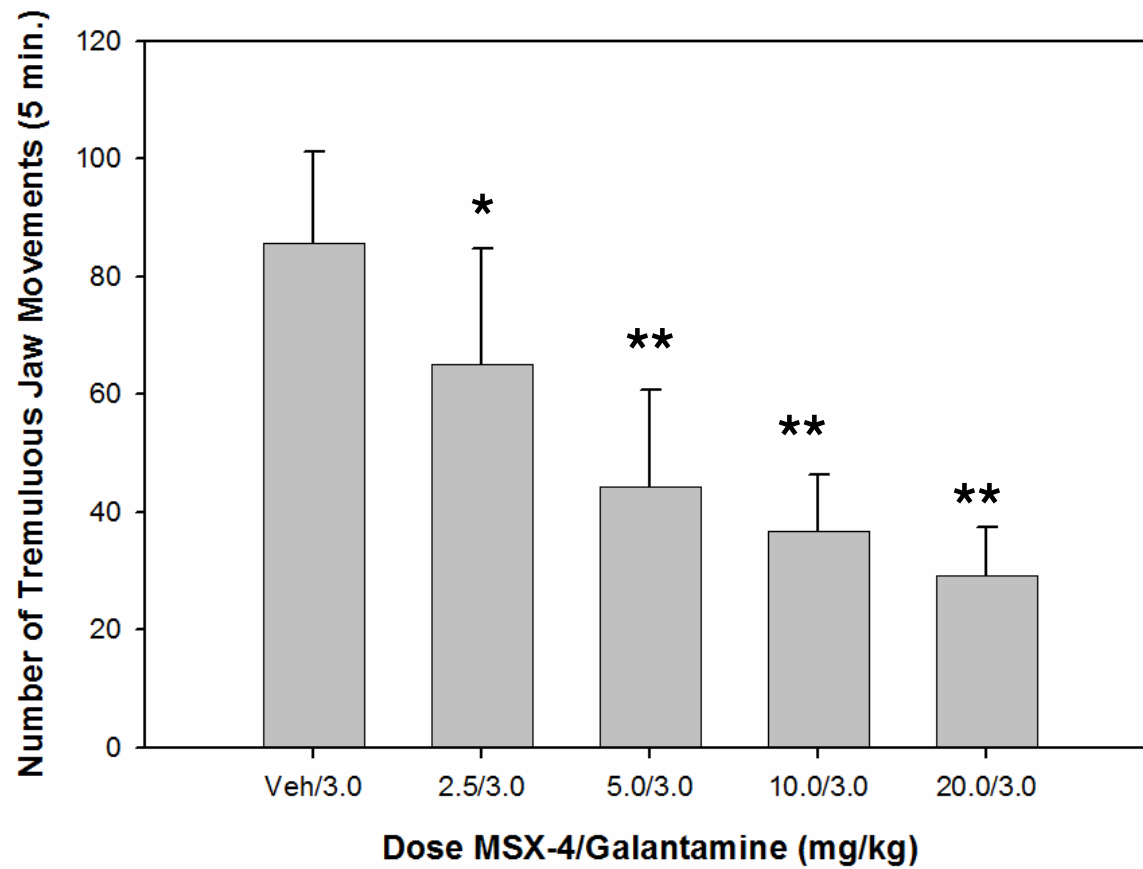

Figure 3