

**Effort, Reward, and Behavior: The Role of A2a Receptors on Effort
Guided Decision Making**

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Abstract

Behavior is guided by motivation; such motivation includes effort and reward. The striatum plays a central role in integrating value of reward and effort associated with behavior, through converging inputs from multiple areas of the brain. Two antagonistic pathways within the striatum are involved in this process; the direct pathway which executes behavior that is rewarded and the indirect pathway which inhibits behavior that is not rewarded or is too effortful. Activation of the direct pathway is aided by increases in dopamine and activation of the indirect pathway is aided by decreases in dopamine. It is thought that A2a receptors have modulatory effects on dopamine signaling in the striatum. However, no study to date has shown the specific effects of A2a receptors in effort-guided behaviors. For this purpose, we developed a new behavioral task to assess effort-guided decision making, in which rats had to choose between two actions that were associated with different amounts of effort. We found that A2a receptor antagonism decreased the sensitivity to effort, but increased ability to assess and adapt to effort changes.

Table of Contents

Abstract.....	2
Introduction.....	5
Motivation	5
Effort and Reward	6
The basal ganglia role in instrumental behavior	8
Striatal activation	9
Dopamine pathways.....	10
The roles of dopamine and adenosine receptors	11
Dopamine’s role in activation of behaviors	13
Adenosine’s role in suppression of behaviors	14
Synergetic activation of the striatum.....	16
Behavioral studies with dopamine and adenosine	17
Experiment and hypothesis.....	17
Materials and Methods	19
Animals	19
Behavioral apparatus	20
Behavioral procedures	20
Habituation	20
Shaping	20
Adaptive Progressive-Ratio schedule	21
Changepoint Environment Task (CPET)	22
Surgery	23
Drugs	23
Histology.....	23
Statistics	24
Results.....	24
Rats adapt appropriately to the task demands.....	24
Analyzing drug effect.....	26

MSX-3 did not increase total number of responses	26
MSX-3 increased response lag at high effort	27
MSX-3 increased probability of switching at high effort.....	29
Discussion.....	30
Conclusion	34

Effort, Reward, and Behavior: The Role of A2a Receptors on Effort Guided Decision Making

There is a link between interactions in the brain and one's behavior, and the goal of behavioral neuroscience is to understand that link. Motivation guides behavior and is influenced by many factors. Through understanding more about motivation, behavior, and the brain, neuroscience can begin to uncover the biological mechanisms that influence behavioral outcomes. However, behavior is quite complex, and cannot be fully explained from an equation of simple motivational constructs in the brain. Yet, taking a small step by trying to understand a single neurotransmitter's role in one aspect of behavior will shed a bit more light on the mysterious universe located inside the brain and how it is linked to behavior.

The goal of this thesis is to understand the neural systems that support effort-guided decision making. To accomplish this I will first describe some of what is known about motivational theory and the neural systems involved in instrumental behavior. Then I will present my hypothesis and experiment regarding the role of striatal MSN A2a receptors in an effort-guided decision making task that we developed.

Motivation

In behavioral neuroscience motivation can be seen as “a set of processes through which organisms regulate the probability, proximity, and availability of stimuli” (Salamone & Correa, 2012). In other words, motivation initiates, continues, and modifies behavior oriented towards a specific outcome or goal. If an outcome is the goal, and behavior is the means to accomplish that goal, then motivation is the drive for the outcome to be achieved through behavioral changes.

Motivated behaviors are influenced by both temporal and qualitative factors (Salamone & Correa, 2012). Temporal factors include either reward seeking or reward taking behaviors. Reward seeking is the process of pursuing rewarding stimuli that includes appetitive, preparatory, instrumental, and approach behaviors (Salamone & Correa, 2012). Reward taking is the process of receiving reward and is primarily described as consummatory behavior (Salamone & Correa, 2012). In addition to temporal factors, there are qualitative aspects that influence motivation. Qualitative aspects of motivation include directional and activational behavior (Salamone & Correa, 2009). Directional behaviors are driven by either seeking rewarding stimuli, or avoiding non-rewarding and painful stimuli (Salamone & Correa, 2009). Activational behaviors determine if the outcome is worth pursuing based on the amount of work, or effort, needed to fulfill it (Salamone & Correa, 2012). From this, effort and reward are aspects that influence motivated behaviors.

Effort and Reward

At least two variables influence goal directed behavior: (a) the value of outcome (pleasurable vs non-pleasurable outcome) produced by the behavior and (b) the effort needed to execute the response (response cost). The influence of reward on behavior has been extensively investigated. Thus, this thesis aimed to understand how response effort contributes to response selection. Specifically this thesis is designed to understand how dopamine and adenosine receptors contribute to the process that enables rats to assess response cost, and use this assessment to adapt flexible to task demands.

Effort is seen as the energy needed to pursue an outcome (Salamone & Correa, 2012). Effort requires learning to occur, in order to assess the cost of the effort. Thus one assess the effort behind each action and determines if it is worth the outcome, and tries to use as little effort as they can to try to achieve the best outcome. One learns to allocate their resources (e.g. , time and energy) to accommodate the effort needed, to receive the outcome of a rewarding stimulus. Decisions based on effort need to have more than one possible response, thus choosing between responses. It is important to appreciate that, before one can make a decision based on response effort, one must first acquire representations of the response costs. For example, for a rodent to decide between two responses of different cost, such as having to press a lever twice (low cost) to obtain a response versus having to press another lever 6 times (high cost) for the same outcome, it must have experience with these response categories and acquire a representation of them.

The experiment in this thesis focuses on the motivations underlying instrumental behavior. Instrumental behavior involves behavior that is repeated due to past results in a particular outcome (Aarts, Holstein & Cools, 2011). We will address instrumental behavior resulting from positive reinforcement. Here, the presentation of an appetitive stimulus (eg. Chocolate milk) following a behavior (eg. Lever response) increases the probability that behavior will reoccur. Instrumental behavior includes both actions and habits. Actions are goal directed behaviors and habits are stimulus-response behaviors (Dickinson & Balleine, 2002; Aarts, Holstein & Cools, 2011). In instrumental behavior, the reward is the reinforcing outcome, and the behavior is the means for achieving that outcome.

The methodology for pursuing an analysis of the neurobiological basis of effort assessment will be presented at the end of the introduction. However, to appreciate the hypothesis that guides the research it is first necessary to provide an overview of the neural system thought to support instrumental behavior.

The basal ganglia role in instrumental behavior

The basal ganglia are thought to play a critical role in generating motor behaviors. However, this region does more than control motor movement, it is also involved in guiding effort, motivation, learning, and ultimately whether any planned motor behavior is executed (Aarts, Holstein, & Cools 2011).

The basal ganglia are a complex neurocircuit consisting of many different brain areas. The primary nuclei of the basal ganglia include the striatum, the globus pallidus, the subthalamic nuclei, and the substantia nigra pars compacta. The striatum is the largest of the basal ganglia and is composed of the dorsal striatum (caudate nucleus, and putamen) and the ventral striatum (nucleus accumbens). Because information from many brain regions converge onto the striatum, it is thought to be the last stop before behaviors are executed. It receives dense input from the cortex, amygdala, hippocampus, and thalamus. The striatum also receives input from the midbrain dopamine neurons in the substantia nigra pars compacta (SNc). These inputs are integrated within the striatum and that information projects out to the globus pallidus and the midbrain centers. The globus pallidus is the major output region of the basal ganglia sending projections to the thalamus (Purves, Augustine, Fitzpatrick, Hall & LaManita (Eds.), 2008).

Striatal activation

Behavioral execution is determined by the activation of medium spiny neurons (MSNs) in the striatum. There are two primary types of MSNs within the striatum that determine behavioral activation, the direct pathway and the indirect pathway MSNs (Schiffmann, Fisone, Moresco, Cunha & Ferre, 2007; Frank, Seeberger & O'Reilly, 2004; Surmeier, Ding, Day, Zhongfeng & Weixing, 2007). Activation of the direct pathway neurons produces motor movement, thus allowing behavior to occur. Activation of the indirect pathway neurons inhibits motor movement, thus preventing a behavior from occurring. These pathways contain projections to and from other regions of the brain, and it is these connections that influence whether or not the direct or the indirect pathway neurons are active and if behavior occurs.

Multiple inputs converge in the striatum and are responsible for its activation or suppression. The largest input to the striatum is the cerebral cortex, which provides excitatory glutamate input. Many different regions of the cortex project to the striatum; these projections are referred collectively as the corticostriatal pathway (Purves, Augustine, Fitzpatrick, Hall & LaManita (Eds.), 2008). The cortex has multiple functions, but for the scope of this thesis, it is responsible for activating the striatum when a behavior is worth pursuing. Particularly, the prefrontal cortex (PFC) is involved in sending that message to the striatum. The PFC is known as the “executive function” center of the cortex. The PFC is thought to assess reward value, cost of effort, and to predict the outcome that will follow behavior (Purves, Augustine, Fitzpatrick, Hall & LaManita (Eds.), 2008). One specific region in the PFC, the anterior cingulate cortex (ACC), assesses cost of effort (Schweimer & Hauber, 2005; Floresco & Ghods-sharifi, 2007). The ACC activates the

ventral striatum (nucleus accumbens) when an effort is worth pursuing (Cardinal, Parkinson, Hall & Everitt, 2002; Schweimer & Hauber, 2005; Floresco & Ghods-sharifi, 2007). Note that other regions of the cortex are involved in striatal activation and determine behaviors such as fine-tuned motor movements (Purves, Augustine, Fitzpatrick, Hall & LaManita (Eds.), 2008).

Dopamine pathways

There are two dopamine pathways project to the striatum that are thought to play a critical role in its activation. First, the mesolimbic pathway originates in a region of the midbrain called the ventral tegmental area (VTA) and sends input to the ventral striatum. This pathway is associated with the prediction of rewards and is activated when reward is not anticipated but received, and inhibited when reward is expected but not received (Nei, Kim, Namburi, & Tye, 2012). The other dopamine pathway is the nigrostriatal pathway which originates in the substantia nigra pars compacta (SNc) and sends input to the dorsal striatum. This pathway is associated with developed motor tasks, actions and habits (Nei, Kim, Namburi, & Tye, 2012; Pauli, Clark, Guenther, O'Reilly, & Rudy 2012) and is activated by the ventral striatum and cortex (Aarts, Holstein, & Cools 2011). In addition, some studies show that the ventral striatum's modulation on the dorsal striatum through the substantia nigra (striatal-nigro-striatal pathway) is involved with the transfer of reward learning to higher developed well learned behaviors (Aarts, Holstein, & Cools 2011; Balleine & Everet 2008)

Activation of striatal cells through cortical inputs has the direct outcome of either initiation or suppression of behavior. Cortical inputs in the striatum are modulated by the

input from mesolimbic and nigrostriatal dopamine pathways. Therefore it is not only cortical activation, but the complex interactions that occur at striatal neurons that determine behavioral responses. But how do the neurochemical interactions at the molecular level influence striatal activation?

The roles of dopamine and adenosine receptors

The neurotransmitter input and receptor activation on striatal MSNs determines the response of the direct and indirect pathways. Striatal MSNs release GABA when activated through depolarization. GABA is an inhibitory neurotransmitter, thus striatal cells provide inhibitory output. The direct and indirect pathway MSNs do not only project different output pathways, they express different receptors. The direct pathway MSNs contain dopamine D1 receptors (D1R) and adenosine A1 receptors (A1R). The indirect pathway MSNs contain dopamine D2 receptors (D2R) and adenosine A2a receptors (A2aR) (Schiffmann, Fisone, Moresco, Cunha & Ferre, 2007; Orru et al., 2011). Overall, activation of MSNs occurs through ionotropic glutamate receptors as well as metabotropic dopamine and adenosine receptors.

Ionotropic glutamate receptors AMPA and NMDA initiate excitement of all striatal MSNs. These receptors increase cellular depolarization which may activate the cell. However, cellular activation depends on the strength of depolarization and needs to reach a threshold. Glutamate may not be enough in order to activate the cell. Metabotropic dopamine and adenosine receptors of MSNs modulate the cell response to glutamate. Moreover, because the direct and indirect pathway MSNs of the striatum express different receptors, dopamine and adenosine modulate the MSNs in different ways.

In the direct pathway MSN, dopamine activates and adenosine inhibits the cell response. When dopamine is released onto the direct pathway MSN, it binds to the D1R and causes activation of the CAMP-protein kinase A (PKA) signaling cascade, which aids in cellular depolarization (Shiflett, & Balleine, 2010). In addition, when adenosine is released in the direct pathway, it binds to the A1R and causes inhibition of adenylyl cyclase (Schiffmann, Fisone, Moresco, Cunha & Ferre, 2007). The inhibition of adenylyl cyclase decreases activation of cAMP, and PKA, thus decreasing cellular depolarization (Surmeier, Ding, Day, Zhongfeng & Weixing, 2007). When neurons in the direct pathway depolarize, GABA is released onto the internal Globus Pallidus (GPi) (Schiffmann, Fisone, Moresco, Cunha & Ferre, 2007). Thus, dopamine binding to D1R enhances GABA release, and adenosine binding to A1R inhibits GABA release. This is significant because GABA release in the GPi will go on to produce a behavior. Therefore dopamine enhances the direct pathway MSNs to produce a behavior, while adenosine suppresses the direct pathway MSNs aids in behavioral inhibition.

In the case of the indirect pathway the roles of the dopamine and adenosine are reversed from the roles in the direct pathway. The indirect pathway is composed of the MSN which express D2R and A2aR. When dopamine is released onto the indirect pathway MSNs, it binds to D2Rs causing inhibition of the adenylyl cyclase, thus decreasing cellular depolarization (Schiffmann, Fisone, Moresco, Cunha & Ferre, 2007; Shiflett, & Balleine, 2010). In addition, adenosine released onto the indirect pathway MSNs, binds to A2aR, which initiates the PKA signaling cascade, thus aiding cellular depolarization (Schiffmann, Fisone, Moresco, Cunha & Ferre, 2007; Shiflett, & Balleine, 2010). Cellular depolarization in the indirect pathway causes the release of GABA at the external Globus Pallidus (GPe), not

the GPi as seen in the direct pathway (Schiffmann, Fisone, Moresco, Cunha & Ferre, 2007). Dopamine binding to D1R inhibits GABA release, and adenosine binding to A1R enhances GABA release. Therefore the roles of dopamine and adenosine are reversed from what is seen in the direct pathway. Ultimately, adenosine enhances activation of the indirect pathway MSNs which will inhibit behavior, and dopamine suppresses the indirect pathway MSNs which will disinhibit behavior.

Dopamine's role in activation of behaviors

Dopamine is a key neurotransmitter in the striatum for motor activation. As previously stated, dopamine activates direct pathway MSNs and suppresses the indirect pathway MSNs. Dopamine from the SNc and the VTA are released for rewarded behaviors, thus aiding in activation of the direct pathway MSNs. The direct pathway MSN releases GABA at the GPi, which is responsible for tonic inhibition of the thalamus (Purves, Augustine, Fitzpatrick, Hall & LaManita (Eds.), 2008). This is important because the thalamus activates the cortical regions which then produce behavior (Purves, Augustine, Fitzpatrick, Hall & LaManita (Eds.), 2008). The GPi tonically inhibits the thalamus, which prevents unintended behaviors from happening. However, when the direct pathway MSNs are activated, GABA released from the MSN inhibits the GPi, thus disinhibiting the thalamus, and allows activation of the cortex to initiate a behavior. Therefore when dopamine is released in the striatum, there is an increased activation of the direct pathway MSNs and behavior is more likely to occur (see figure 1).

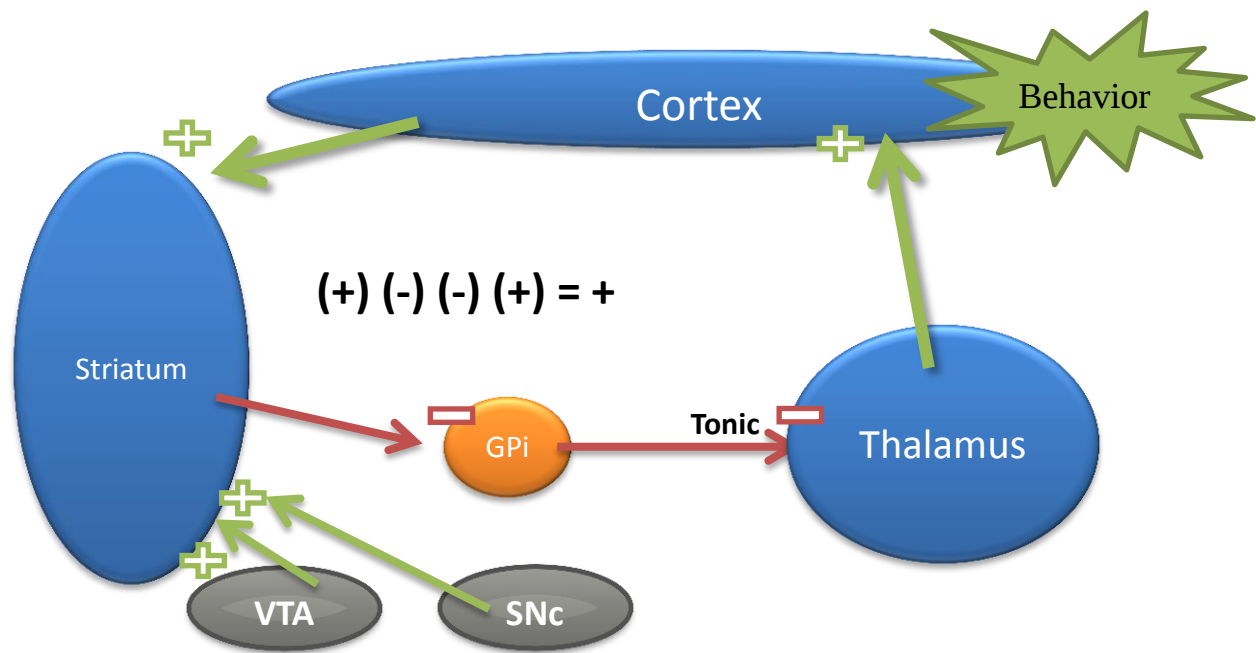


Figure 1. The direct pathway of the striatum is activated by the dopamine from neurons in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) and activated by glutamate from neurons in the cortex. When active the direct pathway of the striatum inhibits the internal region of the globus pallidus (GPi). The GPi is responsible for tonic inhibition of the thalamus, thus when inhibited, disinhibits the thalamus. Disinhibition of the thalamus allows behaviors to occur. When viewed algebraically two positives multiplied by two negatives equals a positive.

Adenosine's role in suppression of behaviors

Adenosine is a key neurotransmitter in the striatum for inhibition of motor behavior. Tonically present in MSN cells, adenosine is produced locally in the cell as a function of cellular workload (Schiffmann, Fisone, Moresco, Cunha & Ferre, 2007). Adenosine increases activation of the indirect pathway MSNs and inhibits activation of the direct pathway. The indirect pathway MSNs releases GABA onto the GPe. The GPe inhibits the subthalamic nucleus, the projections of which activate the GPi (Purves, Augustine,

Fitzpatrick, Hall & LaManita (Eds.), 2008). This relationship can be better understood by beginning at the final output of the circuitry. The GPi inhibits the thalamus, preventing motor cortical activation. The subthalamic nucleus increases activation of the GPi, continuing behavioral inhibition. Now the GPe comes into play. Its output tonically inhibits the subthalamic nucleus, which no longer excites the GPe, and ultimately the thalamus is disinhibited, and produces behavior. However, when the indirect pathway is activated by adenosine, the GPe is inhibited through GABAergic projections from the striatum. This causes GPi to continue inhibiting the thalamus, ultimately inhibiting motor behavior. Therefore when adenosine is present in the striatum, the activation of indirect pathway MSNs is increased, which may ultimately suppress behavior (see figure 2).

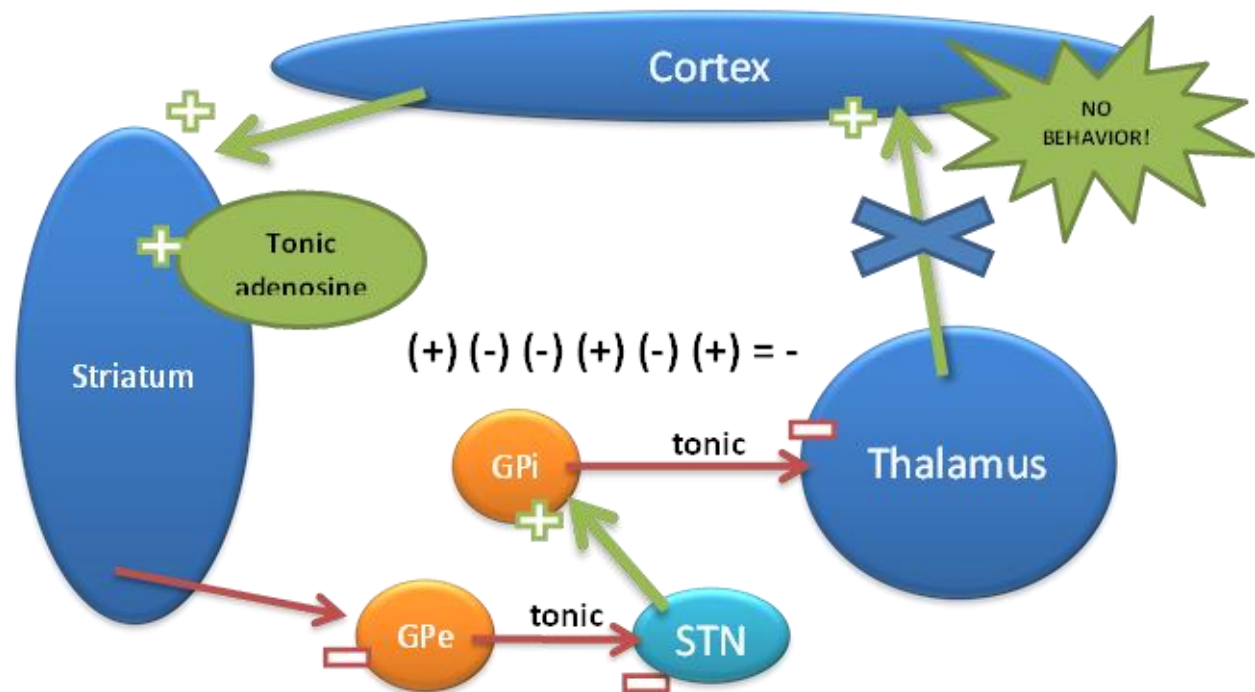


Figure 2. The indirect pathway of the striatum is activated by tonic adenosine and glutamate neurons from the cortex. When active the indirect pathway of the striatum inhibits the external portion of the globus pallidus. The globus pallidus inhibits inhibition

of the subthalamic nucleus (STN) thus the STN is disinhibited. Disinhibition of the STN allows activation of the internal segment of the globus pallidus. With activation the globus pallidus continues tonic inhibition of the thalamus. Inhibition of the thalamus prevents behavior from occurring. When viewed algebraically, three positives multiplied by three negatives, equals a negative.

Synergetic activation of the striatum

As stated earlier, cellular activation depends on the strength of depolarization and needs to reach a threshold. We know that glutamate receptors, A2aR, and D1R are all involved in cellular activation, and A1R and D2R are involved in cellular deactivation. In addition, dopamine is not just released during reward, but is tonically released by the SNr and VTA (Purves, Augustine, Fitzpatrick, Hall & LaManita (Eds.), 2008). This means there are basal levels of dopamine present at striatal synapses. . Pleasurable stimuli are associated with burst of dopamine and increase the likelihood that direct pathway MSNs are activated. In contrast, non-pleasurable stimuli are associated with dips of dopamine and increase the likelihood direct pathway MSNs will be activated. (Salamone & Correa, 2012; Purves, Augustine, Fitzpatrick, Hall & LaManita (Eds.), 2008).

Due to the dopamine pathways, dopamine has a modulatory effect on cortical glutamate released at the striatum. And, as stated before, there are basal levels of adenosine in the striatum. Thus locally present adenosine has a modulatory effect on dopamine in the striatum. With dopamine and adenosine's competing effects, how is it that depolarization is able to occur? It is thought that, not just the presence of dopamine, glutamate, or adenosine that activates MSNs, but it is the amount of each at a given time that synergistically activate the MSNs. All of these systems have to contribute to cellular depolarization for cellular activation to occur (Surmeier, Ding, Day, Wang, & Shen 2007).

Behavioral studies with dopamine and adenosine

Dopamine is thought to be involved in cost/assessment tasks in animals. Decreases in dopamine in the striatum, through dopamine antagonists, have shown to increase sensitivity to effort in T-maze tasks as well as in lever pressing in rat studies. Da antagonists decrease lever pressing for more desirable food, but increased food intake for less desirable food. In addition, in T-maze tasks, Da antagonists increased choices towards low effort arms with less food, and decreased choices towards high effort arms with more food. (Salamone & Correa, 2009).

Also, A2a receptors have been shown to regulate dopamine in effort-guided decision tasks for rats, and A2aR antagonism shows increased responding on rewarded tasks. A2aR agonists have been shown to block the effects of D2 antagonists, and partially block the effects of D1 antagonists, where A1 agonists have not done so in effort-guided choice behaviors in rats (Salamone & Correa, 2012). Also, adenosine A2a receptor antagonists have shown to increase in response rate for lever pressing in high progressive ratio tasks for rats (Randall et al., 2011).

These studies address A2aR effects on rewarded behaviors, dopamine effects on cost/assessment behaviors, or A2aR modulation of dopamine in effort-guided behaviors, however, no study to date has shown the specific effects of A2aR in effort-guided behaviors.

Experiment and hypothesis

Because dopamine is known to affect effortful behavior, and adenosine's modulation of dopamine's effects, adenosine should influence choice behavior as well. Specifically, what are A2aRs effects on effortful rewarded behavior? The purpose of this study is to

address A2a receptors effects on effort assessment in decision making. In this experiment we specifically look into the role of A2aR in lever pressing behavior for an effort-guided decision making task.

To assess effort we developed a new instrumental task for rats called the Changepoint Environment Task (CPET). Rats were trained to press two levers, up to six times on each lever, to gain reward. The lever costs were independent of the other, where one lever cost was less than the other at a given point. The low cost was 2 or 3 lever presses and the high cost was 5 or 6 lever presses. In addition, the reward schedules changed every 8-10 rewards received, or trials. This task is unique from other behavioral tasks because the distinction between low and high effort is very small. This is important because, as seen with progressive ratio schedules, rats switch to habitual behaviors, which greatly increases response rate. A lower response rate in CPET engages the rat to distinguish between low and high effort should keep the rat sensitive to response cost.

To more fully appreciate this paradigm, imagine that the rat is currently pressing the low cost lever (e.g. reinforced for 2 presses). When the rat has completed 8-10 trials that lever now changes to the high cost lever (e.g., reinforced for 6 presses). To behave adaptively, the rat needs to complete the 6 response on the now more effortful lever and then, after being rewarded, it should shift to the other lever, which requires less effort.

CPET requires that the rat (a) continuously monitor its response output and (b) learn that it should shift levers when the required number of responses on the lever it is currently pressing increases. Two measures of performance were taken; (a) response lag—the interval between responses, which assessed the rats ability to monitor output and

(b) the probability of shifting from the more effortful to the less effortful lever, which assessed the rats ability to detect the changes in effort.

Once the behavioral task was learned, the role of A2aRs was assessed in a repeated measures test. Before the test session, MSX-3, an A2aR antagonist, or saline vehicle was infused into the rat striatum. Testing occurred during two days in which the drug treatment was switched for each rat. Thus each rat was tested in the control and drug condition.

We hypothesize that antagonizing A2aRs should inhibit the indirect pathway, causing a disinhibition of behavior. With this “brake” in ongoing instrumental behavior removed, it was expected that MSX-3 treatment would show decreased sensitivity to effort, allowing them to more rapidly complete the response requirement (reduced response lag) on the more effortful lever than control treatment. Completing this response sequence would enable them to more rapidly gather the information needed to determine that the current lever had become more effortful and, upon receiving the reward, to then increase their probability of shift to the alternative lever. Thus, MSX-3 treatment would show increased ability to assess and adapt to the changes in effort in the task.

Materials and Methods

Animals

Twenty three, male, Long Evans rats (Harlan, Indianapolis, IN) were used in the study. The rats weighed an average of 330g. They were put on food deprivation to maintain their weight at 90% of their ad libitum weight, with free access to water. They were housed in a room with a 12-h light-dark cycle. One rat was excluded from the study because it did

not learn to press levers, three rats were excluded because they no longer performed CPET after surgery, and two rats were excluded because their cannulae were damaged.

Behavioral apparatus

Training and testing took place in eight operant chambers containing two response levers (Coulbourn Instruments), placed 5 cm to the right and left of a liquid dipper that delivered 2% reduced-fat chocolate milk (Lucerne Foods) as a reward. Each chamber was housed within a sound and light resistant cabinet. A 3-W, 5-V house light, mounted in the center of the ceiling provided illumination. Experimental protocols were reviewed and approved by the University of Colorado Institutional Animal Care and Use Committee (IACUC).

Behavioral procedures

Habituation

At the beginning of the experiment rats were introduced to drinking chocolate milk. They were placed in home cages with a petri dish containing 15mL of chocolate milk for up to forty-five minutes. This was repeated for up to five days until each rat finished the chocolate milk within 20 minutes.

Shaping

The goal of shaping was to get the rats to learn to press both levers equally often. Initially, rats received five minutes of magazine training, during which chocolate milk reinforcement was given every 20 seconds in addition to reinforcing every lever press (fixed ratio 1; FR1). After a rat had pressed a lever 20 times, this lever was associated with an FR2 schedule. To promote exploration a variable interval (VI) schedule of reinforcement

was combined with the ratio schedule. For each lever the VI followed a uniform distribution. Throughout the experiment the size of the variable interval in seconds was of the same magnitude as the fixed ratio schedule. For example if a lever was associated with a fixed ratio schedule of 3, the maximum of the VI was 3 seconds. To ensure the rats pressed both levers equally often, correction terms were added to the variable interval and the ratio schedule. The correction term added additional time to the VI. For example, if a rat pressed one lever over the other, the difference in total lever presses would be added in seconds to the VI of the favored lever. This would make the rat have to wait additional time on the favored lever to be rewarded again from that lever. For the ratio schedule, the correction term analogously adjusted how many times a rat had to press a lever in order to get rewarded. For example if the rat pressed the right lever 5 times and the left lever 3 times, the rat would not be able to be rewarded on the right lever for 2 seconds (in addition to the VI), and the rat had to press the right lever an additional 2 times. In addition intervening presses of the opposite lever did not cancel already performed presses on this lever during this phase. The rats continued to be trained on this schedule until they pressed each lever at least 100 times and the difference between right and left presses was less than 5 presses.

Adaptive Progressive-Ratio schedule

After Shaping, the rats were trained on a dynamic Progressive-Ratio schedule. Initially the levers required two presses to be rewarded, but throughout the phase rats had to learn to press up to five consecutive times, or 5 times in a row without pressing the other lever, to receive reward. This was achieved by increasing the necessary lever-presses by one, every five rewards. To ensure that rats continued to press both levers in this phase, pressing a

lever was not reinforced if they had received 5 more rewards from this lever than from the other lever. Rats were maintained on this schedule until they had received 25 or more rewards under the final 5 lever-press requirement.

Changepoint Environment Task (CPET)

In the final task, the rats were trained to press a lever up to six consecutive times to get reward. In particular, a lever had to be pressed either 2 or 3 times in a low effort condition, and 5 or 6 times in the high effort condition. If one lever was associated with the high effort condition, the other lever was associated with the low effort condition. For example one lever may require three presses, and the other may require six. The ratio schedule changed every 8-10 rewards. In addition, to promote exploratory behavior, a VI continued to be associated with each lever. As in earlier phases of the study, the maximum VI, in seconds, associated with a lever was the same as the effort assigned on the lever. For example a VI of 2 seconds was associated with a lever cost of 2 presses (see figure 3).

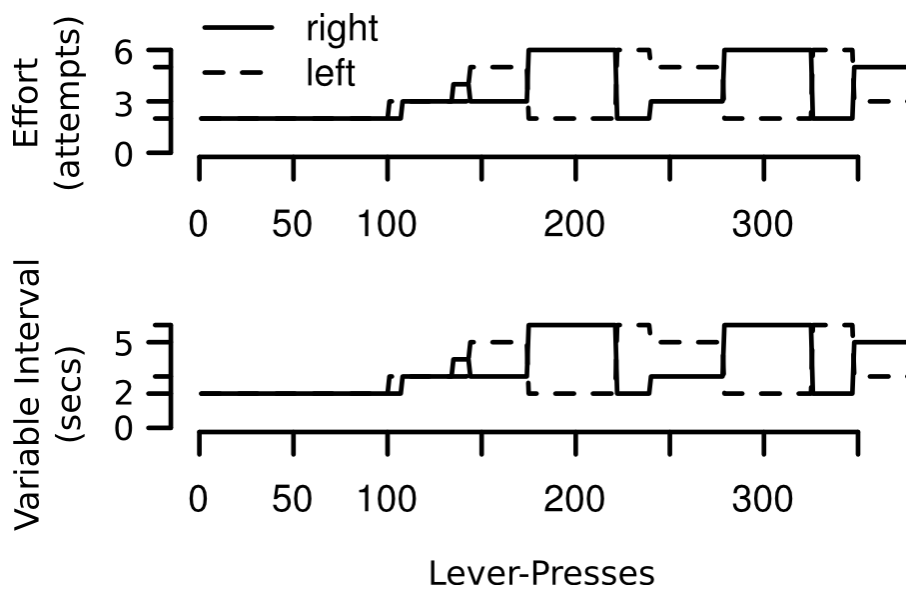


Figure 3. The top graph indicates the effort level on each lever throughout a testing session for one rat. The bottom graph indicates the VI on each lever throughout the testing session for the same rat.

Once a rat had pressed low effort levers significantly more often than high effort levers, for two consecutive sessions cannulae were surgically implanted.

Surgery

The rats were anesthetized with Halothane and stereotactically placed 26-gauge stainless steel guide cannulae (Plastics One) bilaterally into the striatum. Bregma was used as reference for implantation into the striatum following the coordinates anteroposterior: -0.1mm, mediolateral: $\pm$ 3.1mm, dorsoventral: -3.6 mm. After surgery took place, the rats had a 7 day resting period.

Drugs

We injected the A2a antagonist MSX-3 (Sigma-Aldrich; M3568-25MG) bilaterally into the striatum. MSX-3 was dissolved in saline solution with the concentration of 5 ug/uL per side. A saline vehicle served as control. On the first day, half of the rats received MSX-3 and the other half received saline in randomly assigned groups. On the second day of training, the drugs were switched for each rat to allow for a repeated measures test. Ten minutes after injections the rats were tested on CPET.

Histology

When testing was completed the rats were anesthetized with halothane, decapitated, and their brains were taken and frozen in isopentane. Coronal sections were cut (40um thick) through the striatum on a cryostat and mounted every third section.

Slides were stained in cresyl violet and examined by light microscopy to verify the cannulae placement. In this study, all rats that received surgery had correct placement.

Statistics

To measure how well rats adapted to changes in effort we measured the probability of switching to the other lever after reward was received, as well as the response lag (time between two consecutive lever presses). Effort was measured by the number of consecutive lever presses needed for reward on that lever. Probability of switching to the other lever was determined by the number of switch choices over the number of total choices. Response lag, or the time elapsed between each lever press, was determined by averaging the average time between each lever press for each rat.

For statistical support we ran liner mixed-effects models to determine interactions between variables. In these models, subject identity was coded as a random effect. Because drug and effort was controlled for, they were coded as a fixed effect.

Results

Rats adapt appropriately to the task demands

Given CPET was a new task, the first question to answer is –are the animals sensitive to the shift in effort required, that is can they detect change point? The data presented in Figure 4 indicate that they can. It shows that the probability of shifting levers increased as a function of effort level. A liner mixed effects model shows a significant effect ($t(235)= 10.93, p<.001$).

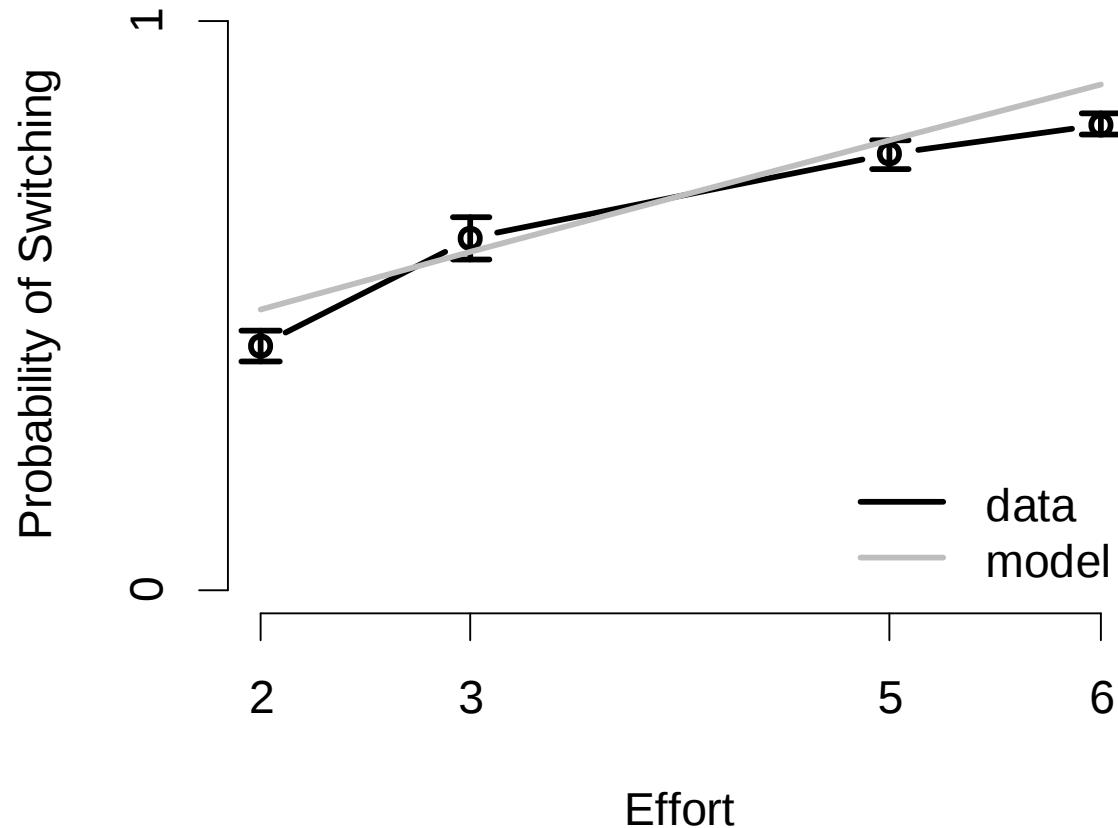


Figure 4. The probability of a rat switching levers increased with effort level. The gray line indicates the slope of the model.

As an example, it is useful to examine in more detail the data from an individual rat. Figure 5 depicts the difference in effort value (left-right) on the two levers (red line) and the subsequent difference in lever preference (right-left) by the rat (black line) during a testing session. The black line generally tracing the red line indicates that the rat switched to the right lever as the difference in effort for the left lever, relative to the right, increased. In other words, as the effort on a lever decreases, the rat's preference for that lever increases. This indicates the rat is detects the changes in effort level on each lever during the task, and is able to adapt to these changes.

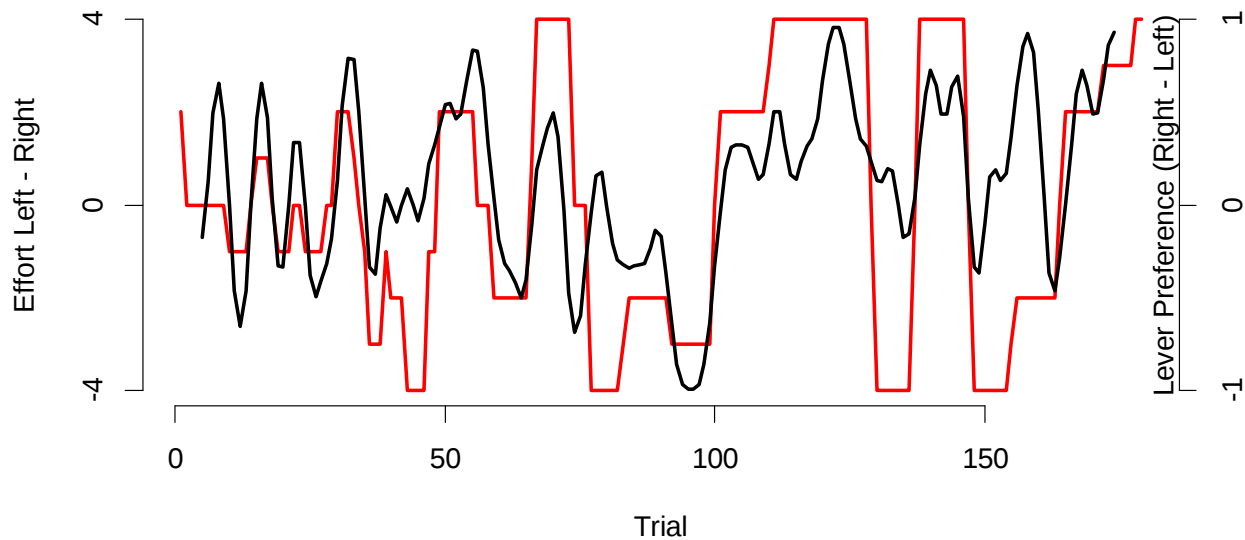


Figure 5. The effort level of the lever is displayed in red, Lever preference is displayed in black. The rat switched to the right lever as the difference in effort for the left lever, relative to the right, increased.

Analyzing drug effect

MSX-3 did not increase total number of responses

When administering a drug it is important to determine if the drug changed overall activity level. In a repeated-measures test, the difference between the lever presses when a given rat received the control solution versus MSX-3 was not significant, control: 160.65 presses, MSX-3: 186.36 presses ($t(16)=1.30$, $p=.23$). In addition, there was no difference between the mean total rewards received by each rat for control versus MSX-3 condition. The mean difference between the drug and control was 12.35 rewards per session, and this was not a significant difference ($t(16)=1.3$, $p=.2112$). Because rats did not display an overall increase in lever pressing or rewards received when treated with control versus MSX-3, differences in response lag or probability of switching are not due to an overall increase in instrumental behavior.

MSX-3 prevented response lag increases at high effort

Next we assessed the response lag, or amount of time elapsed between each lever press, to assess rats ability to monitor output. As is the common, the distribution of response lag times was highly skewed with a long right tail, dramatically increasing the standard errors. Hence, instead of taking the average response lag for each rat, the log response lag was used, because it is known to stabilize the variance reaction time distributions. For ease of visualization, Figure 6 is displayed using seconds, but the reported statistical tests are based on the more stable log response times. Figure 6 displays the average response lag for control and MSX-3 (unbold lines) against the prediction of a linear mixed effects model (bold lines). The unbold lines depict the rats continuing the same response lag until they reach five attempts on a lever. After five attempts on a lever, the response lag increases greatly for the control treatment, and stays the same for the MSX-3 treatment. Figure 7 is the detailed analysis and description of this issue accounting for high effort, using the log of the response lags

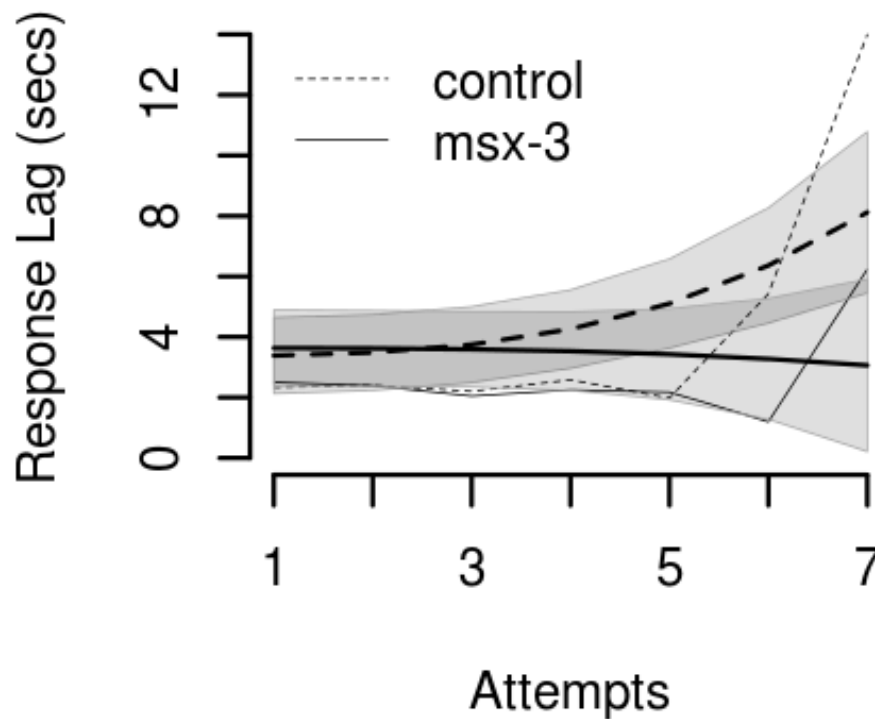


Figure 6. There is no difference in response lag between injection groups at low efforts. Once the effort gets above five presses, there is a difference in response lag where MSX-3 injections decrease response lag and control injections increase response lag.

Although the average difference (1.04 seconds) comparing control trials to MSX-3 trials, was not significant ($t(5879) = -.34$, $p = .73$), there was a significant interaction with effort ($t(5879) = -3.79$, $p = .0002$) such that for the low-effort lever there was no difference in response lag between injection groups, whereas for the high effort lever there was a significant difference in response lag between injection groups.

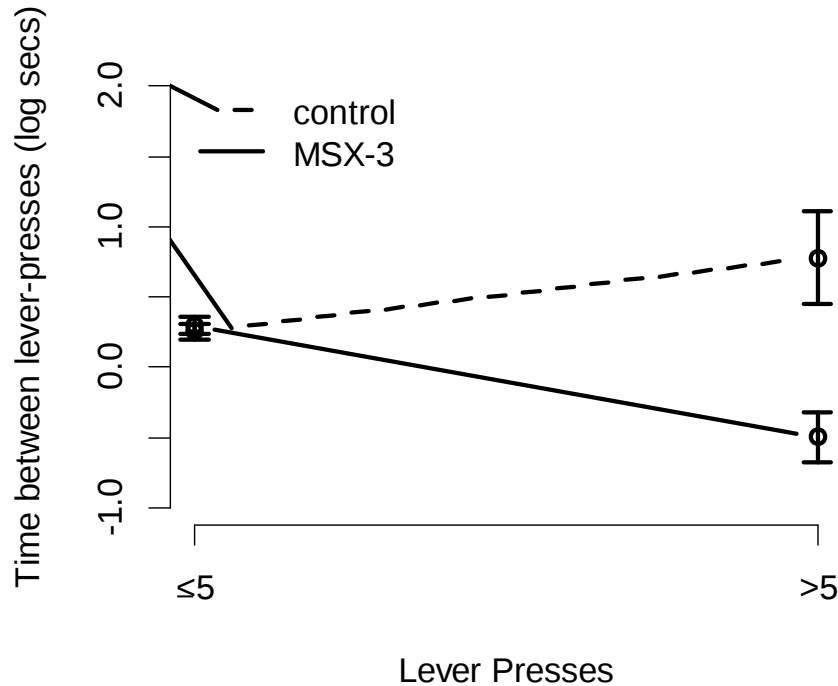


Figure 7. At effort equal to and below 5 presses, the log response lag was not different. At effort above 5 lever presses the log response lag for MSX-3 decreased from low effort, and the log response lag for control increased from low effort.

MSX-3 increased probability of switching at high effort

Next, we analyzed the probability of switching to the opposite lever, in order to address if the rat is picking up on the changes in effort. For the probability of switching, there was a significant interaction between effort and drug. On low effort levers the probability of switching was not different for injection group. On high effort levers, the probability of switching was enhanced by MSX-3 but decreased by the control (see figure 8). This statistic was significant ($t(43)=2.87$, $p=.0063$).

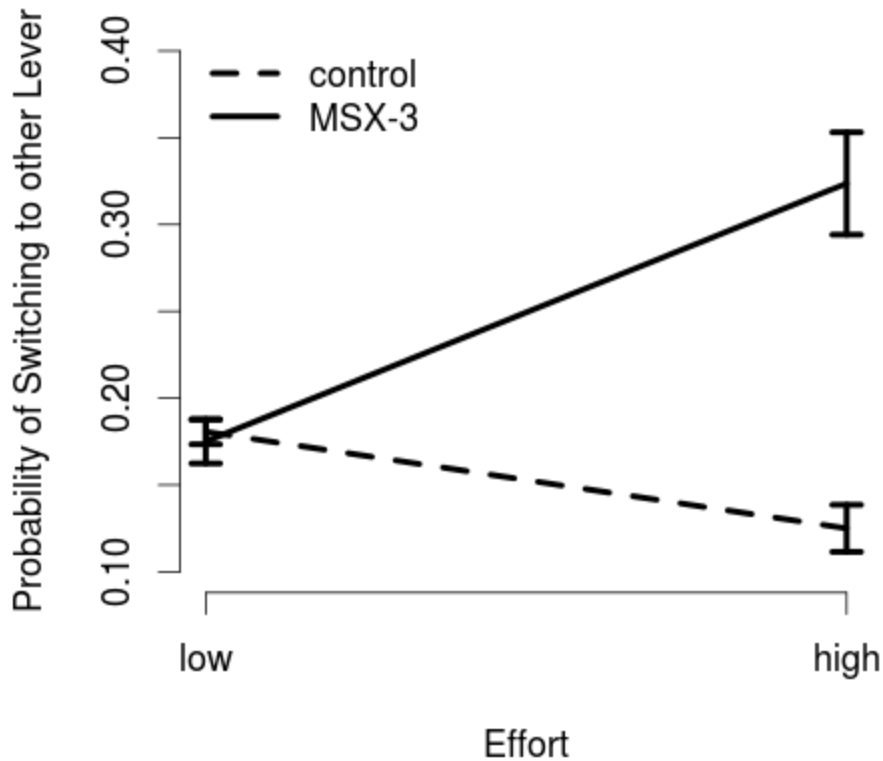


Figure 8. The mean log of response lag for each group at low effort was not different. However, at high effort the mean log of response lag for MSX-3 significantly less than the control.

Discussion

The results from this experiment support the hypothesis that the A2a receptor antagonist treatment decreased the rats' sensitivity to effort and increase their ability to assess adapt to changes effort. Adenosine activates the indirect pathway, and through antagonizing A2a receptors, the "brake" on behavior is reduced. Under MSX-3 treatment, rats show a significant decrease in response lag for high effort levers, compared to the control vehicle. This indicates that A2a antagonism decreased the rats' ability to monitor outcome, thus decreased sensitivity to effort. In addition, following their encounter with

the high effort sequence, rats tested under MSX-3 were more likely to switch to the other lever requiring less effort. MSX-3 treatment allows the rat to adapt to the task better than when under control conditions. This suggests the rats accessed the information needed to guide their switch to the other lever. Thus A2a antagonism increased the rats' ability to assess and adapt to changes in effort.

We found that the probability of switching for the control group decreased from low effort to high effort. It would be expected that from low to high effort the probability of switching would increase, or even stay the same, but not decrease. This may be due to the rat's ability to monitor outcome during the task. The response lag for the control group increased at effort over five lever presses, this indicates that the rats may have been unable to assess the effort on that lever. Since the response lag increased, the rats could have lost the ability to monitor effort level associated with that lever, thus were also unable to adapt to the changes in effort.

These data fit in with current literature. It is thought that dopamine modulates glutamate in MSNs of the striatum (Surmeier, Ding, Day, Wang, & Shen 2007). Also it is thought that A2a receptors have modulatory effects on D2 receptors of the striatum (Salamone & Correa 2009). Because of these receptors complex signaling in striatum and the striatum's role in effort and reward assessment, A2a receptors play a role in these behaviors, as we are able to see in this experiment. When it comes to effort guided decision making, dopamine antagonism is thought to increase sensitivity to effort (Salamone & Correa 2009). Also, A2aR agonists have been shown to block the effects of D2 antagonists in effort-guided choice behaviors in rats (Salamone & Correa, 2012). Our data fit in,

indicating that through modulation of D2 receptors, A2aR antagonism decreases sensitivity to effort.

Although adenosine A2a receptors may play a small role in the bigger picture of the striatal mechanisms that influence behavior, these data have important implications. First of all, understanding how effort and reward is addressed at a molecular level in the brain gives us more information about how the brain influences behavior. Also, it is meaningful to understand how decision making, based on effort and reward, is affected by chemical signaling in the brain. For example, caffeine is an adenosine receptor antagonist. It is a commonly used substance found in a majority of food and beverages. Thus understanding more about A2a receptor antagonists in effort guided decision making gives us a greater understanding about the effects of caffeine on behavior.

Specifically, these data are important for the clinical field regarding Parkinson's disease (PD). PD is a disorder in the SNc, where dopamine neurons are depleted to the striatum (Surmeier, Ding, Day, Zhongfeng & Weixing, 2007). This depletion causes an under-activation of the direct pathway and over-activation of the indirect pathway due to the reduction of dopamine. Thus, the behavioral effects of PD patients include not being able to initiate motor movements, and eventually lead to decreased cognitive ability. One common drug for the treatment of PD is Levodopa, a dopamine agonist. Levodopa helps with the initiation of motor movement through increasing dopamine in the striatum (Ramlackhansingh, Bose, Ahmed, 2011). However, Levodopa also causes unwanted motor tremors, which could be due to under activation of the indirect pathway with changes in striatal output to the GPi (Ramlackhansingh, Bose, Ahmed, 2011). Based on evidence of A2a

receptor modulatory effects on dopamine, A2aR antagonists could be paired with Levodopa treatment to balance out the under activation of the indirect pathway in PD patients taking Levodopa.

In addition, an interesting point to consider is where injections occurred. We injected, into the general region of the striatum, an increased dose that would diffuse throughout the striatum. This was intentional because A2a receptors of the indirect pathway are located throughout the striatum. However, it is hypothesized that the striatum contains functionally distinct regions (Aarts, Holstein & Cools, 2011; Purves, Augustine, Fitzpatrick, Hall & LaManita (Eds.), 2008). To assume A2aR antagonism of the whole striatum would only influence effortful behavior is inappropriate. The striatum is known to influence a wide range of behaviors, motor movements, all the way to cognition (Aarts, Holstein & Cools, 2011; Purves, Augustine, Fitzpatrick, Hall & LaManita (Eds.), 2008). However, since there is still a lot of unknown about the functionally different regions of the striatum, starting at the whole region was beneficial for an initial assessment of the role of A2a receptors.

Because of the theory of distinct functions of different regions of the striatum, a future study could investigate the role of A2aR in varying regions of the striatum. MSX-3 could be injected into the dorsal striatum as well as the ventral striatum to analyze the different roles of A2aR in these regions, and ultimately assess the different functions of these regions have in effort guided decision making. In addition, investigating the modulation of the mesolimbic pathway on the nigrostriatal pathway in effort guided decision making could be addressed.

The story depicted in this thesis about behavior in the striatum may be over generalized and simplistic. Many other interactions occur at the striatal level that have not been discussed. Despite these limitations, the following discusses the most generalized view of motivation and behavior constructed in the striatum to the scope of this thesis.

Because information from any brain regions converges onto the striatum, it is thought to be the last stop before behaviors are executed. The PFC, and particularly the ACC are thought to assess costs of effort. The molecular representation of this assessment is sent to the striatum through ACC release of glutamate to activate MSNs. The mesolimbic and nigrostriatal pathways are thought to assess reward learning and goal directed behaviors. The molecular representation of this assessment is sent to the striatum through dopamine release or dips on MSNs. This dopamine release is modulatory of glutamate release due to metabotropic mechanisms of dopamine receptors. In addition, adenosine modulates dopamine's effects on the striatum due to the metabotropic mechanisms of adenosine receptors. All of these neurotransmitters released at the striatum are involved in one purpose, initiating or preventing behaviors from occurring. It is the changes in tonic and phasic bursts of adenosine and dopamine, as well as the cortical release of glutamate that could be seen to represent motivational aspects of behavior. Thus the striatum determines behavioral output based on the synergistic activation of MSNs through these inputs.

Conclusion

In summary, this experiment investigated the role of A2a receptors in the striatum and in an effort-guided task. Our results supported our hypothesis that A2aR antagonism in

the striatum decreases sensitivity to effort and increases ability to assess and adapt to changes in effort. By contributing to our understanding about behavior, motivation, and the brain, neuroscience can begin to uncover the biological mechanisms that influence behavioral outcomes. This is beneficial in society in order to understand oneself, others and our relations to our environments. In addition, it also gives us insight into understanding and solving problems related to psychological disorders.

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