The role of the Adenosine A3 subtype receptor in time perception

The ability to perceive time is important for the survival of many organisms, including humans. Without this ability, behaviors such as cooking or crossing a street could lead to unfortunate outcomes. In fact, any type of motor coordination and many types of perceptual abilities, including auditory and visual perception, rely on temporal discrimination. Many factors (e.g., mood, psychological disorders, drugs, etc.) have been shown to reliably improve or disrupt accuracy in timing as well as speed up time perception or slow it down (Meck, 1996). However, the effects that caffeine, the world's most consumed psychoactive drug, has on temporal perception are not clear. Over the last several years, I have worked with Dr. Neval Erturk and over a dozen students conducting experiments to attempt to answer this question. Below, I summarize the research that has been done on caffeine and temporal perception and then propose a follow up experiment to determine how caffeine, through specific adenosine receptors, affects temporal perception.

Temporal perception can be influenced along two, somewhat independent dimensions: accuracy and bias (i.e., speed of a hypothetical internal clock). To illustrate the difference, imagine you are asked to estimate the duration of a light that was turned on for 60 seconds. Practice at estimating various durations with feedback will likely help you improve your accuracy. Conversely, drinking lots of alcohol before this task will likely reduce the accuracy. With regard to bias, taking methamphetamine, or other stimulants, will likely "speed up your internal clock" and thus, if a stimulus was on for 60 seconds, you might experience those 60 seconds as 90 seconds. Conversely, taking haloperidol, or other depressants, will likely "slow down your internal clock" and you may estimate the 60 second stimulus to have lasted 40 seconds. As a stimulant, caffeine should speed up the internal clock. In our previous studies summarized below, we have found this result.

Caffeine is an adenosine antagonist (Solinas et al., 2002). Adenosine affects a wide range of neuropsychiatric functions primarily integrating with dopaminergic and glutamatergic neurotransmitter systems. There are four adenosine subtype receptors: A1, A2A, A2B, and A3, with each having a variety and sometimes opposite functions (Shen & Chen, 2009). Caffeine binds to all four subtype receptors, blocking adenosine from functioning properly. For example, the gradual accumulation of adenosine during waking hours results in tiredness, but ingesting caffeine blocks these effects and makes one more alert. Due to the distribution of the four subtype receptors in the brain, and the co-localization of some adenosine receptors with dopaminergic receptors (a neurotransmitter widely studied for its role in temporal perception; Cevik, 2003), understanding which adenosine subtype receptor is involved in temporal perception is very important in understanding the neurobiology of timing behavior.

Previous Findings

As noted above, I (working with Dr. Neval Erturk and several research students) have conducted several studies to: 1) determine how caffeine affects temporal perception and 2) determine which specific adenosine subtype receptor is implicated. Figure 1 summarizes several experiments using various doses of caffeine using a Stubbs' Timing Procedure. In the figure, data points above zero indicate a speeding up of temporal perception while data points below zero indicate a slowing down of temporal perception. As can be seen, low and moderate doses of caffeine speed up temporal perception, peaking at approximately 20 mg/kg. However, there is a bi-directional effect. As the dose of caffeine becomes large enough, the speeding up of temporal perception is eliminated and a slowing of temporal perception is observed. This bi-directional, dose-dependent effect of caffeine has been documented previously (Ferre et al., 2007; Gruber & Block, 2003).

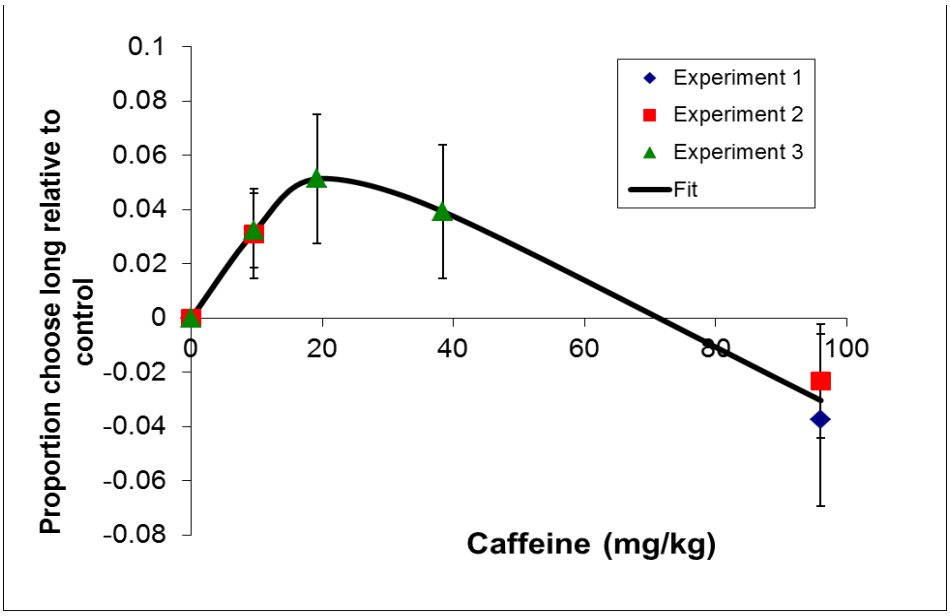

Figure 1. Three experiments which varied the dose of caffeine are summarized. Data points above 0 indicate a speeding up of temporal perception while points below indicate a slowing down of temporal perception.

After establishing that caffeine can speed up temporal perception, we then conducted a study to determine which specific adenosine subtype receptor is implicated. In the initial study, we gave different groups of rats specific adenosine subtype receptor antagonists. Thus, we had a sham control group, as well as A1, A2A, A2B, and A3 antagonist groups. Figure 2 illustrates the outcome of this study. As can be seen, only the A2A condition showed a speeding up of temporal perception similar to what was observed in Figure 1 when using small and moderate doses of caffeine.

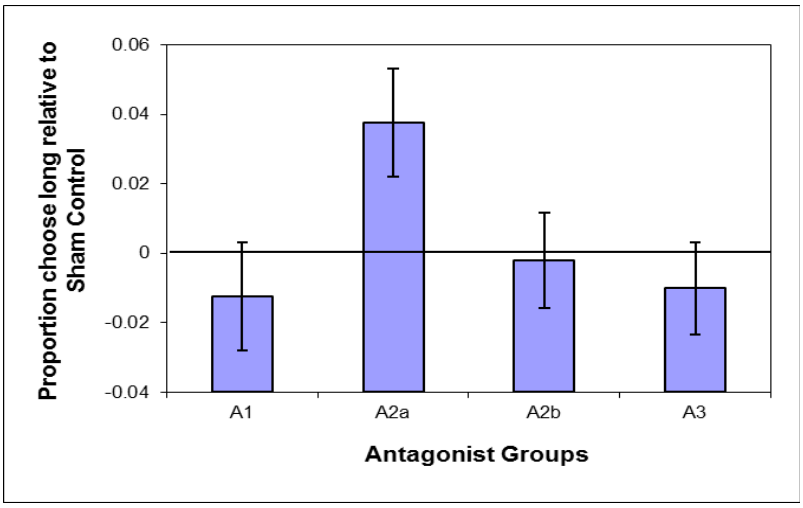

Figure 2. The results of a study are displayed in which adenosine subtype receptors were blocked through the administration of selective antagonists. Shifts above 0 indicate a speeding up of temporal perception. The A2A condition mimics the caffeine conditions shown in Figure 1.

When comparing the results illustrated in Figures 1 and 2, it is clear that A2A is indicated in its role in temporal perception. However, we still cannot rule out the other three subtype receptors. As discussed above and as can be seen in Figure 1, caffeine has a clear, bi-directional dose dependent effect. It is possible that the lack of effect observed in the three other groups is simply due to the dose used. To rule out each subtype

receptor, we conducted follow up studies using multiple doses of each subtype receptor antagonist. As expected, the study using various A2A antagonist doses confirmed the role of the A2A subtype receptor (see Figure 3). Experiments that varied the doses of A1 and A2B resulted in further evidence that they do not play a role in the effects of caffeine on temporal perception. No A1 or A2B dose was significantly different from the Sham Control Group. However, we ran out of funds before we could conduct the final study in this line of research, that of testing the A3 antagonist at various doses. Thus, I am submitting this proposal in hopes of gaining funds to complete the final experiment needed in this line of research.

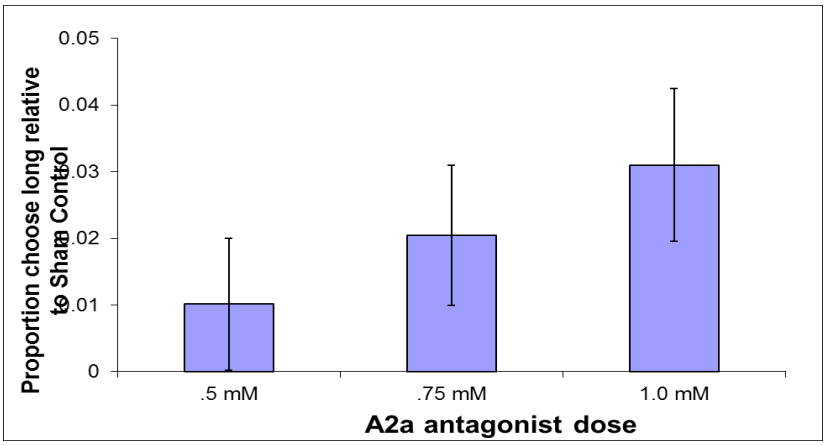

Figure 3. The results of a study that varied the doses of A2A antagonists.

Procedure

In order to determine if the A3 subtype receptor is implicated in the effects of caffeine on temporal perception, I will use 24 rat subjects on a Stubbs timing procedure (Cevik, 2003). The experiment will consist of two phases: a training phase and a testing phase. The purpose of the training phase is to train the rats to discriminate between short and long signals in Skinner boxes. For example, the short signal could be associated with the left lever and the long signal could be associated with the right lever (short and long levers will be counterbalanced across rats). Short signals will include signals less than four seconds and long signals will be signals over four seconds. Training should take approximately two months.

After the discrimination is learned, testing will begin using the four different treatment conditions: a sham control group, and low, medium, and high dose experimental groups (2.5, 5, and 7.5 µM of 3-Ethyl-5-benzyl-2-methyl-4-phenylethynyl-6-phenyl-1,4-(+)dihydropyridine-3,5-dicarboxylate, an A3 selective antagonist). I will use a within subjects design so each rat will undergo all conditions in a counterbalanced order (see Table 1). Testing will take 18 consecutive days. Treatments will be administered via intraperitoneal (IP) injections, which include direct injection into the abdominal cavity. This method of administration results in precise control of dosage and rapid absorption into the bloodstream. Each condition will continue for three consecutive days, followed by two days of no injection, followed by the next condition for three consecutive days, etc.

Data Analysis

Data will be analyzed using a repeated-measures ANOVA in SPSS. The main dependent measure will be the degree of bias in choosing the long versus the short lever. Choosing the long lever more (relative to the sham control condition) indicates faster temporal perception while choosing the short lever more indicates slower temporal perception. Secondarily, a repeated-measures ANOVA will be used to determine if accuracy in discrimination between the short and long signals is affected by the different conditions. In addition, the data

from the proposed study will also be compared with the previous studies using various doses of selective antagonists.

Table 1. The counterbalanced order of the testing phase is displayed for each rat.

Rat #	Phase 1	Phase 2	Phase 3	Phase 4
1	Sham	Low	Medium	High
2	Sham	Medium	Low	High
3	Sham	High	Low	Medium
4	Sham	Low	High	Medium
5	Sham	Medium	High	Low
6	Sham	High	Medium	Low
7	Low	Sham	Medium	High
8	Low	Medium	High	Sham
9	Low	High	Medium	Sham
10	Low	Sham	High	Medium
11	Low	Medium	Sham	High
12	Low	High	Sham	Medium
13	Medium	Low	Sham	High
14	Medium	High	Low	Sham
15	Medium	Sham	High	Low
16	Medium	High	Sham	Low
17	Medium	Low	High	Sham
18	Medium	Sham	Low	High
19	High	Medium	Low	Sham
20	High	Sham	Medium	Low
21	High	Low	Sham	Medium
22	High	Medium	Sham	Low
23	High	Low	Medium	Sham
24	High	Sham	Low	Medium

References

Cevik, M.O. (2003). Effects of methamphetamine on duration discrimination. *Behavioral Neuroscience, 117,* 774-784.

Ferré, S., Agnati, L. F., Ciruela, F., Lluis, C., Woods, A. S., Fuxe, K., & Franco, R. (2007). Neurotransmitter receptor heteromers and their integrative role in 'local modules': the striatal spine module. *Brain research reviews*, *55*(1), 55-67.

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Shen, H.Y. & Chen, J.F. (2009). Adenosine A2A receptors in psychopharmacology: Modulators of behavior, mood and cognition. *Current Neuropharmacology, 7*(3), 195-206. doi: 10.2174/157015909789152191

Solinas, M., Ferre, S., You, Z.B., Karcz-Kubicha, M., Popoli, P., & Goldberg, S.R. (2002). Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. *Journal of Neuroscience, 22*(15), 6321-6324.

2016 INBRE UNDERGRADUATE STUDENT/FACULTY RESEARCH PROGRAM

FACULTY ENDORSEMENT/QUALIFICATIONS FORM
(Approximately 500 characters per section)

1. How will you document student hours spent on the project, work performed by the student(s), and report the information to INBRE?

   I will use the standard work study time sheets which will be reviewed on a weekly basis. Further, because of the nature of running subjects in Skinner boxes, it will be obvious if the student researcher was not there or even late for the daily experiments.

2. How will you support the student(s)?

   I will train the students in all behavioral techniques used in the experiment and I will recruit Dr. Erturk to train them in giving ip injections. During data collection and after, I will train the students on data analysis and work with them in both scientific oral and written reports.

3. What learning outcome(s)/goals do you expect your student(s) to achieve?

   The students will become proficient in nearly every aspect of a behavioral and physiological study. They will learn how to collect data using MedPC software, become proficient in SPSS software to conduct statistical analysis, and to improve both their written and oral communication skills. Both students have plans to attend graduate school in psychology and this opportunity will both improve their chance of getting in as well as make them more prepared once they start their graduate career.

4. Please list your qualifications to support the project and the student(s) involved.

   I earned a PhD from Indiana University in Psychology, specializing in Animal Learning and Behavior. After earning my PhD, I worked for three years as a post doc at Brown University researching temporal perception in rat subjects. For the last twelve years, I have supervised nearly 20 research students on a variety of projects, with over half of them using rat subjects. My students have given close to 20 presentations at regional and national conferences

   Richard Keen

   FACULTY SIGNATURE

2016 INBRE UNDERGRADUATE STUDENT/FACULTY RESEARCH PROGRAM

STUDENT ACTIVITY/RESPONSIBILITY FORM

(To be completed by the student/s involved in the research project. Approximately 500 characters per section)

1. What are your qualifications to conduct the project?

   Jessica McGivern and Kathleen Langbehn are the two students I would like to have on this project. Jessica is junior psychology major and has been my research assistant for the past year working on a different caffeine experiment using human participants. She has had a semester of handling rats in my Psychology of Learning lab class. In the spring, she will take the Statistics and Research Methods class from me which will further make her prepared for this project. Kathleen is sophomore psychology major and has also been my research assistant for the past year working on the same human, caffeine experiment with Jessica. Kathleen has also had my Psychology of Learning class and will also be taking my Statistics and Research Methods class in the spring. Both students have the necessary personality for this project. They are both bright and persistent and they have been a pleasure to work with this past year.

2. What learning outcome(s)/goals do you hope to achieve?

   As mentioned above, they will learn how to collect behavioral data, give ip injections, analyze and report data both in a written and oral format. They both hope to present this experiment at a regional or national conference.

2016 INBRE STUDENT/FACULTY UNDERGRADUATE RESEARCH PROGRAM
BUDGET JUSTIFICATION FORM

TOTAL BUDGET: $19,000 = $4,000 (experimental materials) + $4,500 x 2 (student stipends) + $6,000 (faculty stipend)

Student stipends:
In order to complete the experiment before the end of the semester, both students will need to work 40 hours per week as we will have to work weekends. I have 5 Skinner boxes in my lab which then limits us to running 5 rats at a time. Each session will last nearly 1 ½ hour per rat with time in between rounds to switch the rats, and eventually when testing occurs, in giving injections and waiting 15 minutes for absorption. Thus, we will run rats around 10 hours per day, seven days a week. With two students each working 40 hours each week, this totals 80 hours per week. The overlapping 10 hours per week will be for multiple lab meetings each week as well as weekly cleaning of the lab. I have already talked to the students about working on weekends and they are ok with it.