Effects of Caffeine on Prospective and Retrospective Working Memory in Rodents

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Introduction:

Trapold (1970) found that, in a biconditional discrimination task, subjects who were trained with unique and distinct outcomes following each discriminative stimulus-response (S-R) sequence acquired the task in significantly fewer trials than those subjects for whom only one outcome was employed. This training procedure, referred to as differential outcomes (DO), is shown in Figure 1, along with the more traditional common outcomes (CO) procedure where only one outcome is employed, or a nondifferential outcomes (NDO) procedure where two outcomes are employed but the outcome presented after each S-R sequence is random.

Figure 1: training procedure for differential outcomes

This improvement in performance, called the differential outcomes effect (DOE) is also seen across delays as an improvement in working memory; that is, subjects trained under DO perform with greater accuracy across delays, even at delay intervals where subjects trained under CO or NDO are performing at near chance levels. This DOE is strong enough to allow subjects to overcome the effects of amnestic drugs and lesions designed to mimic the effects of Korsakoff's syndrome (Savage, 2008).
The difference in performance may be due to the separate procedures engaging different forms of memory. To solve a choice task under CO or NDO, subjects must remember the discriminative stimulus presented at the beginning of the trial using retrospective memory. However, we theorize that subjects under DO develop outcome-specific expectancies of the specific outcomes associated with each sample and it is these prospective memories of what is to come (rather than memory of what has already happened) that guides behavior on any given trial (Holden & Overmier, 2015). These retrospective and prospective codes may well be mediated by different memory systems in the brain, dependent on different classes of neurotransmitters and different areas of the brain (e.g. frontal lobes and limbic system). Our laboratory has conducted a series of pilot studies examining how a number of drugs linked to memory influence behavior under DO and NDO in the hopes of establishing neurochemical similarities and differences between the two systems.

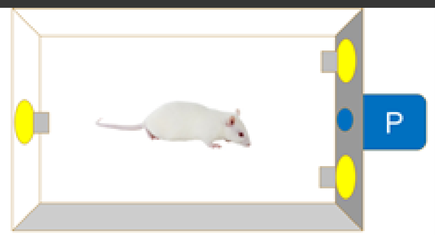
Methylxanthine caffeine is a psychostimulant drug most widely consumed by people today as part of their daily routines due to its desired effect of keeping us awake and functional, which is the result of its role as an antagonist to the adenosine receptors (Angelucci, Cesário, Hiroi, Rosalen, & Da Cunha, 2002; Dubroqua, Low, Yee, & Singer, 2014). Adenosine is a naturally occurring cellular chemical that has been implicated to affect levels of general fatigue during time spent awake, higher amounts being associated with drowsiness and lower amounts associated with wakefulness. By inhibiting adenosine receptors, caffeine can trigger the release of norepinephrine and affect areas of the brain containing these receptors, such as the CA2 region of the hippocampus, the anterior cingulate cortex, the medial prefrontal cortex, the basolateral amygdala, and the mesolimbic dopaminergic areas – all of which may have a role in certain types of memory and consolidation (Borota, Murray, Keceli, Chang, Watabe, Ly, Toscano, & Yassa, 2014; Favila & Kuhl, 2014).

Previous studies have reported results that conflict with one another as to how and to what degree caffeine affects different aspects of memory. Angelucci et al. (2002) found that caffeine administration in rats after being previously trained in a habituation task improved their memory consolidation, and that administration before training was ineffective and suggestive of a null effect on working memory and acquisition. Another study found that participants consuming only smaller amounts of caffeine performed significantly poorer compared to those in the control group in a test of memory recall using 15-word lists of common nouns (Terry & Phifer, 1986). Many studies examining the effects of caffeine on memory tend to focus on acquisition, retention, and consolidation, but few seem to explore working memory. The present study investigates the relationship of caffeine on prospective and retrospective working memory with the prediction that caffeine will significantly lower performance levels overall in the delayed matching-to-position task for both the DO and NDO groups when compared to their respective control groups, but that the DO groups will perform better than those in NDO due to the DOE.

Methods:

Subjects were 15 male Sprague-Dawley rats, approximately 4 months old at the beginning of the study. Subjects were housed under a reversed 12:12 light:dark cycle with lights off at 1000, with water available freely. Subjects were reduced to 85% of their free-feeding weight shortly before training began. Subjects were magazine-trained and autoshaped to press the two retractable levers, and hand-shaped to press the fixed/back lever before beginning the matching-to-position task.

Figure 2. Depiction of the operant chamber setup (right = front, left = back). P = pellet feeder. Front levers are retractable, back lever is fixed in place.



Matching-to-Position:

Matching-to-Position: Sessions ran for 80 trials. At the beginning of each trial, the stimulus above either the left or right lever is illuminated and that lever is extended into the chamber; this is the discriminative stimulus. Two responses on the illuminated lever have the effects of extinguishing this light, retracting the lever, and illuminating the light over the back wall lever. For the trial to progress, the subject must then turn to the back wall lever and press. (This is done to ensure subjects do not bridge a delay period by merely remaining in front of the correct lever.) The first response after a 1-second delay period leads to the extinguishing of the back light and the illumination of both left and right lever lights.

The subject's task is now to press the same lever that was illuminated in the first part of the trial. Correct responses are rewarded with either a) three sucrose pellets accompanied by illumination of the feeder light and a 1 sec train of 8 clicks/second from the clicker (the "large" outcome) or b) three 0.5 sec flashes of the feeder light, followed by a single pellet (the "small" outcome). For subjects in the DO group (n=8), each stimulus-response sequence was consistently followed by a specific outcome (e.g. left-left-small & right-right-large or left-left-large & right-right-small). For subjects in the NDO group (n=7) Incorrect responses lead to a repeating of the trial; three incorrect responses in a row leads to a repeating of the trial, but with only the correct lever illuminated at the end of the trial (a forced choice procedure). Only the initial choice on each trial is included in overall calculations of accuracy.

Once subjects learned this task to criterion (3 consecutive days at 85% accuracy or above), they were switched to a delayed version of the task, where the delay period between the illuminating of the back wall light and the time when the trial could be advanced was set to 1, 5,10, or 20 seconds on any given trial. After meeting criterion on this task (3 straight days of 85% or above at 1-second delay and 70% or above at 5-sec delay), subjects began drug testing. In a project conducted just before this one, subjects were exposed to two doses of scopolamine (0.3 and 0.6 mg/kg) and one of saline; at least 48 hours had elapsed since any injections had been given, before testing in the current project began.

Drug Testing:

Order of drug/control administration was counterbalanced across subjects. Subjects were first administered an intraperitoneal injection of caffeine (Sigma Aldrich, St. Louis, MO) dissolved in saline, at a dose of 10 mg/kg, or saline alone, 30 minutes before testing in the delayed-version of the task. After an approximately 48-hour interval, the second treatment was administered (e.g. if saline was administered on the first testing day, then caffeine was administered 48 hours later, or vice versa).

Results:

Figures 3A and 3B shows accuracy on testing days as a function of group, delay, and drug dose condition, for DO and NDO groups respectively. A mixed-design ANOVA showed a significant effect of group, $F(1,13)=35.196$, $p<.001$, a significant effect of delay, $F(3,39)=44.633$, $p<.001$, a significant effect of drug dose, , $F(1,13)=11.262$, $p<.001$, a significant delay x group interaction, $F(3, 39)=27.03$, $p<.001$, a nonsignificant dose x group interaction, $F(1,13)=3.387$, $p=.089$, a nonsignificant dose x delay interaction, $F(3,39)=1.189$, $p=.326$, and a significant group x delay x dose interaction, $F(3,39)=.37$, $p=.775$.
Pairwise Comparisons (Within-Subject): For the DO group, pairwise comparisons using Fisher's LSD found differences between the caffeine and saline conditions at 5 and 10 seconds delay, $t(7)=3.211$ and 1.930, $p=.008$ and .043. For the NDO group, pairwise comparisons using Fisher's LSD found differences between caffeine and saline conditions at 5 seconds delay, $t(6)=2.976$, $p=.013$. * indicates significant difference from saline ($p<.05$).
Pairwise Comparisons (Between-Subject): Pairwise comparisons using Fisher's LSD found differences between DO and NDO in the saline condition at 5, 10, and 20 seconds delay, t(8.994c, 6.36 c, 13)=2.258, 2.258, and 4.444, p=.025, .031, and .001, respectively, and in the caffeine condition at 5, 10, and 20 seconds delay, t(6.905 c, 7.096 c, and 13)=2.368, 3.801, and 6.561, p=.025, .004, and p<.001, respectively.
c indicates df values from a corrected version of the test with equal variances not assumed.

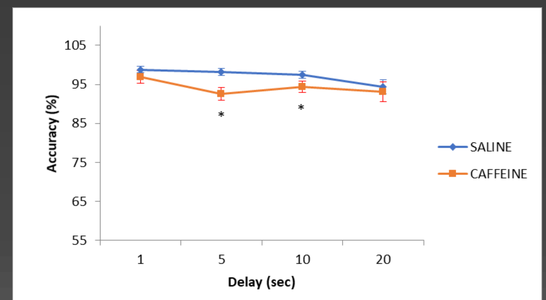


Figure 3A: accuracy on testing days as a function of delay and drug condition for the DO group.

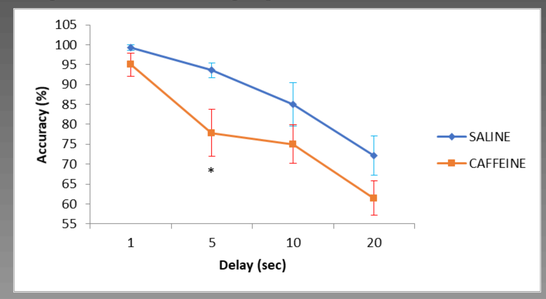


Figure 3B: accuracy on testing days as a function of delay and drug condition for the NDO group.

Discussion:

As expected, the DO group performed more accurately across delays than the NDO group, confirming the DOE. We found that both groups were affected by caffeine administration at the middle delay intervals but not at the longest interval. This may suggest that performance levels of those affected by caffeine administration perform better in the 1-second interval since the delay wasn't too long so that our subjects became distracted, and that longer intervals may cause distraction due to the high levels of physiological arousal caffeine has been known to cause. Another avenue worth exploring is dosage; it was found by Angelucci et al. (2002) that a positive effect on memory consolidation was observed in lower doses of caffeine (0.3 & 3 mg/kg), but not in higher ones (10 & 30 mg/kg). Further research using a larger subject pool and varying doses of caffeine administration would be ideal to more closely observe its effects on prospective and retrospective working memory, and further research is currently being planned for our lab in the near future.

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