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Effects of Caffeine and Caffeine-Associated Stimuli on the Human Startle Eyeblink Reflex

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ANDREWS, S. E., T. D. BLUMENTHAL AND M. A. FLATEN. *Effects of caffeine and caffeine-associated stimuli on the human startle eyeblink reflex.* PHARMACOL BIOCHEM BEHAV **59**(1) 39–44, 1998.—An experiment was performed (n = 19) that investigated the effect of caffeine and expectancy of caffeine on the eyeblink component of the startle reflex. Nineteen habitual caffeine users received caffeinated coffee, caffeinated juice, decaffeinated coffee, or decaffeinated juice in four sessions spaced 1 week apart. Twenty-five to 30 min after ingestion of the liquid, 30 acoustic startle stimuli were presented. The results showed that caffeine increased startle eyeblink amplitude. Startle reflex onset latency was significantly longer in the decaffeinated coffee condition than in the other three conditions. This may have been due to the activation of a compensatory slowing of the reflex by the anticipation of caffeine, a slowing that was then overridden by caffeine speeding the response. © 1998 Elsevier Science Inc.

Caffeine Startle Conditional response Expectancy Placebo Human

THE present experiment investigated the effects of caffeine and conditional stimuli associated with the administration of caffeine on the acoustic startle reflex eyeblink in caffeine users. The stimuli associated with the ingestion of coffee are reliably associated with the effects of caffeine, through the process of classical conditioning, where the effect of caffeine is the unconditional stimulus. This has been termed pharmacological conditioning (29), and has been demonstrated with caffeine in humans. For example, caffeine users show an increased preference for novel drinks that are repeatedly associated with caffeine than for drinks that have never contained caffeine (21).

Pharmacological classical conditioning of antagonistic and agonistic responses may have implications for the processes of drug tolerance and sensitization, respectively. It has been hypothesized (28,30) that drug tolerance is mediated via antagonistic conditioned responses that decrease the effect of the drug. When tolerance occurs, patients and drug users need larger doses of the drug to produce optimal drug effects, and this could lead to increased side effects. Drug sensitization, on the other hand, has been hypothesized to be mediated via conditioned agonistic responses that increase the effect of the drug (28). Such agonistic responses could be viewed as placebo effects (12) that could be beneficial to the drug user.

Caffeine is a central nervous system stimulant with effects on several response systems (6,13,31), including startle. The startle reflex is elicited by stimuli with sudden onset and moderate to high intensity (2), and involves changes in several response systems, including blinking, raising of the shoulders and arms, crouching of the knees, and orienting towards the startling stimulus (18). The blink component is probably the most used index of startle in human studies, because of its reliability and relative ease of measurement. Among the few studies that have investigated the effects of caffeine on startle, Ward, Pollare, and Geyer (37) found increased tactile startle in rats, and Schicatano and Blumenthal (24) found delayed habituation of acoustic startle blink amplitude in both low and high caffeine users, when the subjects received caffeine compared to placebo. Schicatano and Blumenthal (25) showed that this delay of habituation varied with the dosage of caffeine, with delayed habituation when subjects were given doses of 2 or 4 mg/kg, but not at a 6 mg/kg dose.

Few studies have investigated responses to caffeine-accompanying stimuli, and whether such responses may modulate the response to caffeine. Caffeine increases salivation, but tolerance to this effect develops (38). Rozin, Reff, Mark, and Schull (23) crossed ingestion of coffee or apple juice with administration of caffeine or an inactive agent, and measured

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the amount of salivation in 24 habitual coffee drinkers. The results showed decreased salivation to decaffeinated coffee, whereas there was an increase in salivation to apple juice containing caffeine, indicating that an antagonistic response that suppressed salivation was elicited by the conditional stimulus, i.e., ingestion of coffee.

Antagonistic responses to drug-associated stimuli have been observed in some experiments (12,27,30), but not in other experiments (36), and the role of conditioned antagonistic responses in tolerance development is still uncertain. Therefore, the design used in the present study allowed the separate observations of conditioned responses to drug-associated stimuli, unconditioned drug responses, and their interaction. Caffeine users received orange juice with or without caffeine, and coffee with or without caffeine, with the presence of caffeine being administered in a double-blind fashion. Orange juice without caffeine constituted the baseline, whereas orange juice with caffeine should give information about the effect of caffeine without any conditioning or expectancy effects. Responses to decaffeinated coffee should give information about conditional responses, and responses to the caffeinated coffee should give information about the interaction of caffeine with conditional responses.

The present experiment involved only subjects that used the drug habitually. Earlier studies where drug-associated cues have been presented to drug users have found antagonistic responses to opiate- (8), ethanol- (34), and caffeine-associated stimuli (23). There is also some evidence of antagonistic responses to nicotine-associated stimuli (9). Staiger and White (34) found agonistic responses to ethanol-associated stimuli upon presentation of a drink cue. Studies on responses to cocaine-associated stimuli have yielded inconsistent results (4,22). To summarize, in drug users, conditioned antagonistic responses to drug-associated stimuli have been found in many cases. If the stimuli associated with drinking coffee activate a set of compensatory conditional responses, antagonistic to the responses activated by the caffeine itself, then responding in the decaffeinated coffee condition should be opposite to responding in the caffeinated juice condition. Also, responding in the caffeinated coffee condition should be attenuated compared to responding in the caffeinated juice condition.

METHOD

Subjects

Nineteen subjects (9 female and 10 male; avg. age at first session = 19.4 years) were chosen from an introductory psychology class to participate in five sessions 1 week apart on the basis of their responses to a 34-item questionnaire indicating high caffeine intake. High caffeine intake was defined as more than 250 mg of caffeine per day (two to three brewed cups of coffee, or four to five glasses of soda or iced tea) (1). Of these subjects, 11 consumed their caffeine in coffee, and 8 consumed their caffeine in forms other than coffee.

Stimuli

The startle stimulus was an 85 dB broadband noise (20 Hz to 20 kHz), with a duration of 50 ms and a rise/fall time of 0.1 ms. The intertrial interval ranged between 15–25 sec, with a mean of 20 sec.

Materials

The caffeine solution was 100% anhydrous caffeine (Carolina Biological Supply) dissolved in distilled water. Each subject received 4 mg/kg of caffeine. The placebo solution consisted of flat tonic water, which has a bitter taste similar to the caffeine solution, so the subjects would not be able to tell the two solutions apart. We have tested the caffeine and tonic water solutions for discriminability in previous studies, and find that subjects operate at a chance level when taste is used to try to identify the solution. Each of the solutions was mixed in decaffeinated instant coffee or in a powdered orange drink. The fluid per body weight was identical for all four conditions (caffeinated and placebo coffee, caffeinated and placebo orange drink).

Apparatus

The stimuli were created with a Coulbourn S81-02 noise generator, gated through a Coulbourn S84-04 rise/fall gate, then a Coulbourn S82-24 audio mixer amplifier, to a pair of Telephonics (TDH-49P) headphones. The stimulus intensity was calibrated by emitting a continuous stimulus from the headphones and placing a headphone and coupler on a Quest Electronics 215 sound level meter.

Miniature Ag/AgCl electrodes measured the periorbital EMG activity of the reflex eyeblink response. The raw EMG was sent to a Coulbourn S75-01 high-gain bioamplifier/coupler, where it was filtered (passing 90–250 Hz) and amplified. The signal was then sent to a Coulbourn adjustable gain amplifier, and then to a Coulbourn S76-01 contour-following integrator with a 10-ms time constant. The integrated signal was digitally sampled by a MacPacq MP10 interface every millisecond for 500 ms following stimulus onset, and was then stored in a Macintosh SE microcomputer.

Response latency, amplitude, and probability of the eyeblink response were the dependent measures for each trial. Response onset was defined, based on a slope change algorithm in the scoring program, as the point at which the slope of the EMG record began to noticeably and consistently increase above baseline, with this increase continuing for at least 5 ms without reversal. Response latency was defined as the time from the onset of the stimulus to the onset of the response; response amplitude was the difference between the onset of the response and the peak of the response, measured in arbitrary units and then converted to microvolts; response probability was the percentage of trials on which a response was detected, given that a response could have been detected (no excessive movement artifact). When scoring the data, three possible outcomes were entered: a response occurred during the 100-ms window after stimulus onset; a "zero" trial, indicating that there was no scorable response; or a "bad" trial, meaning that a blink might have occurred but there was too much noise in the response signal to see a response (due to subject movement, or the occurrence of a blink before stimulus onset). To guard against measuring a spontaneous eyeblink when scoring data, an eyeblink was included if its onset occurred 20–100 ms after stimulus onset.

Procedure

All procedures were approved by the University's Institutional Review Board. Subjects attended five sessions 1 week apart; however, there were two weeks between the third and fourth sessions for 12 of the subjects due to Thanksgiving holiday. Throughout the experiment the subjects were unaware that the coffee would contain caffeine on only one occasion or that the orange drink would contain caffeine on one occasion. The subject was asked to refrain from ingesting caffeine for at least 12 h prior to each session. To control for time of day effects and help maintain compliance with not ingesting caffeine

prior to attending each session, the subjects were run at approximately the same time each week, in the late morning and early afternoon. The first session was designed to desensitize the subjects to the laboratory setting. During this session the subject went through each aspect of the experiment but was not exposed to the startle stimuli or the caffeine or placebo solutions. The order of solution presentations in the next four sessions was counterbalanced across subjects. Each subject received coffee with caffeine, coffee without caffeine, orange drink with caffeine, and orange drink without caffeine. The solutions were administered in a double-blind fashion, in that neither the subject nor the experimenter knew whether the solution contained caffeine.

The protocol for each of the five sessions was as follows: upon arrival at the lab, the subject signed an informed consent form and filled out a background questionnaire. Then the subject was weighed and given the state portion of the State and Trait Anxiety Inventory (STAI) (33). The experimenter weighed the subject and calculated the fluid per body weight so that each subject received 4 mg/kg of caffeine in caffeine sessions, or an equal volume of tonic water, and mixed either the placebo or caffeine solution into the orange drink or coffee. After the subject completed the STAI he/she was instructed to drink the orange drink or coffee solution as quickly as possible. In humans, caffeine is fully absorbed within 30 min after oral ingestion (14). Twenty minutes after ingestion the subjects were prepared for electrode placement. The experimenter cleaned beneath the subject's eye with a cotton swab saturated with rubbing alcohol and placed two recording electrodes beneath the eye, one electrode below the pupil on the skin overlaying the orbicularis oculi and the other just lateral to the first. A ground electrode was place on the medial surface of the left forearm. The subject was then taken into a sound-attenuated chamber, the procedure was explained, and the subject then put on the headphones and completed another STAI. On all but the first session, stimulus presentation began 25–30 min after solution ingestion and lasted for approximately 10 min, for 30 trials of broadband noise pulses. When stimulus presentation was completed, the electrodes were taken off and the subject filled out a posttest questionnaire, assessing overall affect, and a third STAI. The subject was not fully debriefed until after the fifth session.

Data Analysis

Startle blink response amplitude, latency, and probability were averaged in blocks of three trials, yielding 10 data points for each subject for the 30 habituation trials. Data were pooled across trial blocks and analyzed (BMDP4V) with Greenhouse-Geisser corrected degrees of freedom used to determine significance. For these analyses, there was one between-subjects variable, coffee user (yes or no), and two within-subject variables, caffeine (present or absent) and solution (coffee or orange drink). Orthogonal trend analyses (BMDP2V) were also conducted, to evaluate habituation across trial blocks. In these analyses, there was one between-subjects variable, coffee user (yes or no), and three within-subjects variables, caffeine (present or absent), solution (coffee or orange drink), and trial block (1 through 10). All significant effects found with the 4V analyses were also found with the 2V analyses, so only the results of the 2V analyses will be reported.

RESULTS

For response amplitude, a significant effect of caffeine was found, $F(1, 17) = 4.67$, $p < 0.05$, with caffeine increasing response amplitude (see Fig. 1). A significant effect of habituation across trial blocks was also found, $F(9, 153) = 7.88$, $p < 0.001$, with linear and quadratic trends reaching significance, $F(1, 17) = 10.39$ and 8.63, respectively, $p < 0.01$. No significant effect of either coffee use or solution was found, nor were any interactions significant.

For response latency, a marginally significant effect of caffeine was found, $F(1, 17) = 4.25$, $p < 0.055$, and the caffeine by solution interaction was significant, $F(1, 17) = 5.74$, $p < 0.05$ (see Fig. 2). This interaction was due to the fact that response latency was slower in the decaffeinated coffee condition than in the other three conditions, with these other three conditions not differing from each other. No significant effects of coffee user or trial block were found, in either main effects or interactions.

For response probability, no significant effects were found, although a nonsignificant tendency towards higher probability of responding in the presence of caffeine was indicated. Response probability ranged from 75 to 80% in the four caffeine solution conditions.

State anxiety questionnaire scores did not differ significantly as a function of caffeine, solution, or time during the session. In the present study, caffeine had no effect on state anxiety as measured with the STAI questionnaire.

DISCUSSION

Startle blink response onset latency was not affected by the presentation of caffeine, as can be seen in Fig. 2 by comparing

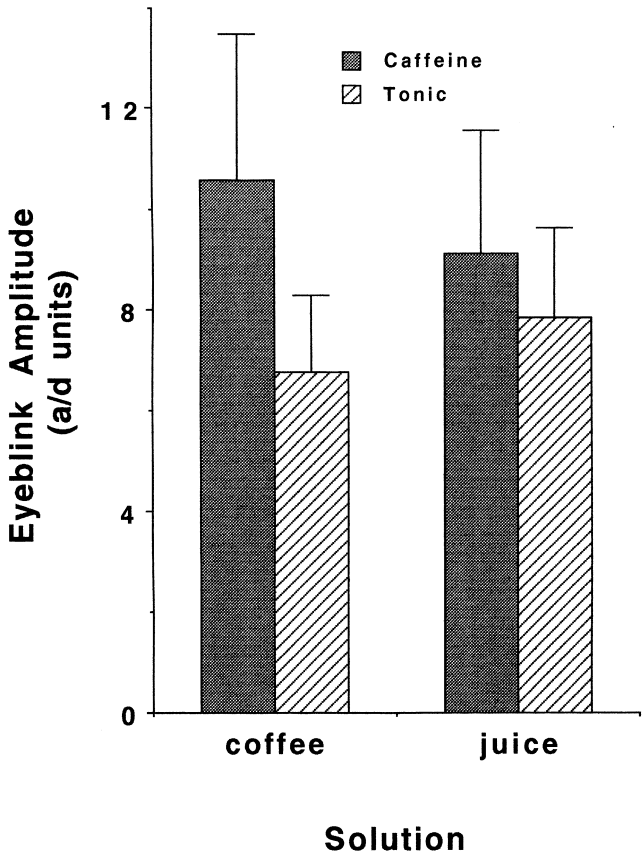

FIG. 1. Eyeblink response amplitude (in analog-to-digital units) as a function of caffeine and solution (bars represent 1 SEM). Caffeine significantly increased response amplitude in both solution conditions.

the tonic-juice condition (which is the baseline against which the other conditions are compared) with the caffeinated juice condition. Previous studies have also shown no effect of caffeine on startle blink response latency, when giving subjects juice either with or without caffeine (24,25). When caffeine was presented in coffee in the present study, it also had no effect on response latency. However, when subjects were given decaffeinated coffee, their startle blink response onset latency became slower, compared to either the tonic-juice condition or the caffeinated coffee condition. This suggests that the expectation of caffeine without the delivery of caffeine results in slower startle blink responding. In the caffeinated coffee condition, subjects both expected and received caffeine, and response latency was at the same level as in the baseline condition. Comparing the two coffee conditions suggests that stimuli associated with caffeine slow responding, and that caffeine speeds responding. The unconditional effect of caffeine overrides the conditional slowing, and this speeds startle blink reflex latency back to its optimal level.

It may be the case that a ceiling effect is present in the juice conditions, in that this latency may be as fast as can be expected for eliciting stimuli with the parameters of those used in the present study. Therefore, startle blink response onset latency may demonstrate a direct effect of caffeine and an antagonistic effect of the expectancy of caffeine. This is in line with the findings of a compensatory conditional response to caffeine-related stimuli reported by Rozin et al. (23). This is a reasonable conclusion if one thinks of a drug effect driving a system away from equilibrium, and a conditional response as bringing the system closer to equilibrium. If the conditional response occurs before the direct drug effect, then the conditional response causes a deviation from equilibrium, and the direct drug effect reestablishes that equilibrium. This latter case is found in the latency data of the present study.

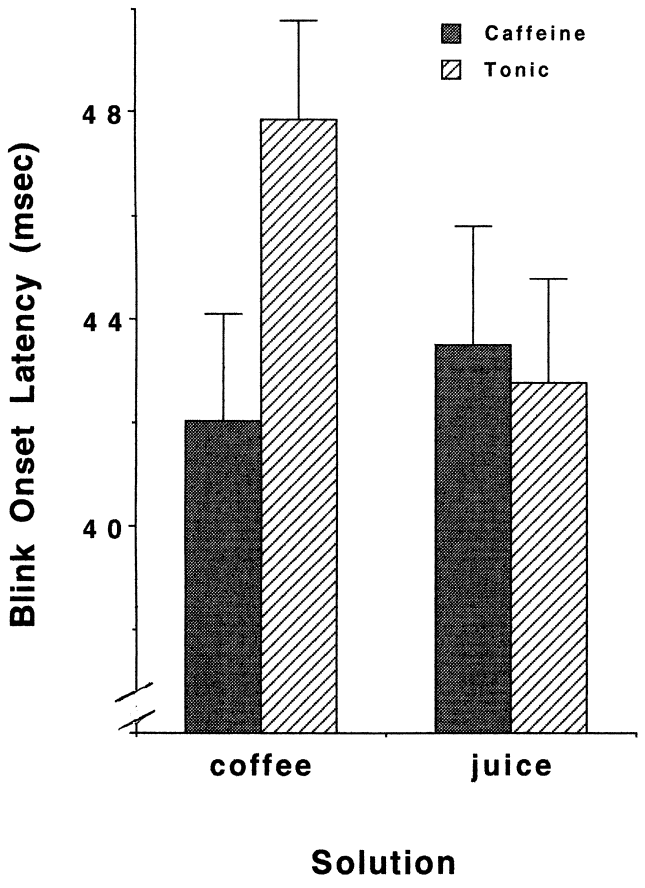

FIG. 2. Eyeblink response latency (in ms) as a function of caffeine and solution (bars represent 1 SEM). Administration of decaffeinated coffee significantly slowed responding.

In the present study an apparently antagonistic CR was observed in the decaffeinated coffee condition, but this CR had no effect on the unconditioned drug response in the caffeinated coffee condition. To our knowledge, this has not been observed in previous studies. The lack of an effect of the CR on the drug response could be due to the relative strengths of the conditioned and unconditioned responses. Four mg/kg of caffeine is a relatively large dose, comparable to about three cups of regular coffee. Thus, the CR elicited in the present study may not have been of sufficient amplitude to antagonize the drug response. Consequently, future research investigating the effect of conditioning on the effect of caffeine should use a lower dose of caffeine.

Previous research (11) has indicated that, when expectancy regarding the effects of caffeine are induced in subjects who receive placebo, behavior that matches the expectancy occurs. Thus, subjects who received decaffeinated coffee and expected to do well on a task did better than subjects who had no specific expectations or who expected to do worse on the task. There are, however, important differences between the present study and previous studies that have induced expectations about drug effects through verbal information. In the present study, a conditioning process that has involved repeated pairings of coffee drinking and the effects of caffeine was studied. When specific expectations are induced through verbal information, the subjects often respond according to the information (3,17,35). However, when the subject has received experience with the drug through a classical conditioning procedure, antagonistic conditioned responses are often, but not always, observed (12,27). Why sometimes agonistic and sometimes antagonistic conditioned responses are observed is still an unsolved problem, but there seems to be a tendency for antagonistic conditioned responses to occur, at least in humans, when the subject has had extensive experience with the drug (8,23,24).

The present results indicated that caffeine increased startle blink reflex amplitude, similar to the findings of Ward et al. (37) with rats, showing overall increased startle amplitude in the presence of caffeine. There was no evidence of a conditional response in the blink amplitude data. Previous research has shown that caffeine increases arousal, as seen in elevated skin conductance responses (29) and cortical ERPs (20), and this could explain its effect on startle amplitude. Findings of increased startle to negatively valent stimuli [see review in (19)], after aversive classical conditioning (32), and to threat of shock (15), support the idea that negative emotional states increase startle. In the present study, startle blink amplitude habituated across trial blocks, but this habituation was not affected by caffeine. Schicatano and Blumenthal (24,25) found delayed startle blink habituation in the presence of caffeine, an effect that was not replicated in the present study. One methodological difference between the present study and that of Schicatano and Blumenthal (24,25) was the use of a familiarization session one week before any data were collected in the present study. In another study, Schicatano and Blumenthal (26) have shown that the impact of caffeine on habituation of startle is not found if subjects are instructed to direct

their attention to a visual task, away from the acoustic startle stimulus. It may be the case that the familiarization session used in the present study altered the attentional demands of the testing situation, although further research is necessary to determine the exact nature of this attentional relationship.

James (16) asked whether the effect of caffeine on caffeine-deprived subjects was due to a net effect of caffeine, or to a reversal of caffeine deprivation. All subjects in the present study were caffeine deprived for at least 12 h, which may generate symptoms of withdrawal [reviewed in (6,14)], including anxiety. Several studies have found faster startle under conditions related to anxiety, fearfulness, and increased arousal (5,15). Thus, according to James'(16) argument, shorter startle latency could be expected during caffeine withdrawal, and this could partly explain the ceiling effect seen in startle blink latency. Thus, administration of caffeine and caffeine withdrawal both seem to speed up startle eyeblink latency, probably through the mechanism of increased arousal. Startle eyeblink amplitude was facilitated by caffeine, which indicates that the degree of arousal induced by caffeine was greater than the degree of arousal induced by caffeine deprivation. Because the subjects in this study had been deprived for about 12 h, withdrawal effects had not yet peaked (10,14), and more pronounced effects on arousal could have been expected with a longer deprivation period. However, slower startle blink latency was seen when the subjects received decaffeinated coffee. This suggests that a compensatory conditional response, opposite to the effects of anxiety, fearfulness, and arousal, was elicited by caffeine-related stimuli. Consequently, when the subjects received decaffeinated coffee, two antagonistic processes may have been at work: effects of caffeine withdrawal, which should speed startle blink responding, and the compensatory conditional response to caffeine-accompanying stimuli, slowing startle blink responding. A large literature indicates that autonomic arousal can be conditioned in humans (7). Caffeine acts to increase arousal, and a compensatory response to caffeine-accompanying stimuli could thus be a decrease in arousal, delaying startle blink response onset.

In summary, caffeine increased startle blink amplitude but had no effect on startle habituation. Slower startle blink latencies were seen when subjects received decaffeinated coffee, which could indicate that caffeine-associated stimuli elicited a response antagonistic to the effect of caffeine itself.

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