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# Effects of Caffeine on Tactile Startle in Rats

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WARD, H. W., T. POLLARE AND M. A. GEYER. *Effects of caffeine on tactile startle in rats*. PHARMAC. BIOCHEM. BEHAV. 15(6) 875–877, 1981.—The effects of graded doses of caffeine (1.5 to 100 mg/kg) on tactile startle responding were examined in rats given 121 air-puff stimuli at 15 second intervals. Doses of caffeine up to 50 mg/kg were found to significantly increase tactile startle consistently throughout the 30 minute test session. The highest dose of caffeine tested initially increased and subsequently decreased startle amplitudes. An examination of the time-course of the augmentation in startle produced by 12 mg/kg caffeine revealed that the effect was maximal 15 minutes after the subcutaneous injection. The results are discussed in relation to the differences between the effects reported here for caffeine and the known effects of other psychostimulants.

Caffeine Tactile startle Reactivity Stimulants

CAFFEINE is one of the most widely consumed psychoactive drugs, yet only recently has it been studied experimentally. Twenty to 30 percent of the United States population consumes 500 to 600 mg of caffeine per day, while ten percent consume more than a gram per day [10]. As with other psychostimulants, most animal studies of the behavioral effects of caffeine have relied on some measure of the locomotor activation typically produced by such drugs. Like amphetamine, methylphenidate, and cocaine, caffeine produces a dose-dependent increase in locomotor activity [13]. However, in contrast to these other stimulants, caffeine produces little of the so-called "stereotyped behaviors" so evident with medium to high doses of amphetamine-like drugs [5].

Doses of d-amphetamine that produce focussed stereotypy in rats (2.5+ mg/kg) also markedly increase startle reactivity in response to either acoustic or tactile stimuli ([1] and references therein). Although lower doses of amphetamine produce dramatic hyperactivity, they do not affect startle reactivity [1,6]. To further define the behavioral effects of caffeine and its similarity to other psychostimulants, our experiments were designed to characterize caffeine's effect on behavioral reactivity as reflected by the rat startle response to tactile stimuli. Measures of startle responding in animals have been used extensively in studies of hallucinogens and other psychoactive drugs [1,7]. Startle measures provide quantitative indices of the level of behavioral reactivity to sensory stimuli and the rate and extent of behavioral habituation. In contrast to amphetamine, our results indicate that even low doses of caffeine significantly increased startle reactivity in rats.

## METHOD

### *Animals*

The animals were experimentally naive male Sprague-Dawley rats weighing 250–300 g on receipt from Hilltop Laboratories (Scottdale, PA). They were housed in pairs on a 12/12 hour light/dark cycle with water and Purina Rat Chow for at least six days before testing.

### *Drugs*

Caffeine (anhydrous) was dissolved in 0.9% saline by gentle warming on the day of the experiment. Control animals received 1 ml/kg of the vehicle. Drug-treated rats received the same volume of the appropriate drug concentration. All injections were given subcutaneously in the middle of the animal's back.

### *Apparatus*

The startle response was monitored in two stabilimeters, as described previously [7]. A phonograph cartridge was used to detect movements of each cylindrical Plexiglas stabilimeter. The stabilimeters were enclosed in a sound-attenuated and ventilated cabinet. A microcomputer based on the Intel 8080 controlled stimulus presentations and recorded each response as 250 readings taken at one msec intervals though an analog-to-digital converter beginning at stimulus onset. The difference between the minimum and maximum readings on a 1000 point scale was taken as the amplitude of each response. Tactile stimuli consisted of 40 msec air-puffs at ten psi delivered though a 1/4 inch pipe directed at the animal's back.

### *Procedure*

Animals were brought to the laboratory at least one hour prior to testing and assigned randomly to groups. For each experiment, groups were counterbalanced for time of day and for stabilimeters. At the appropriate time after injection (usually 15 min), each rat was placed in a stabilimeter and the

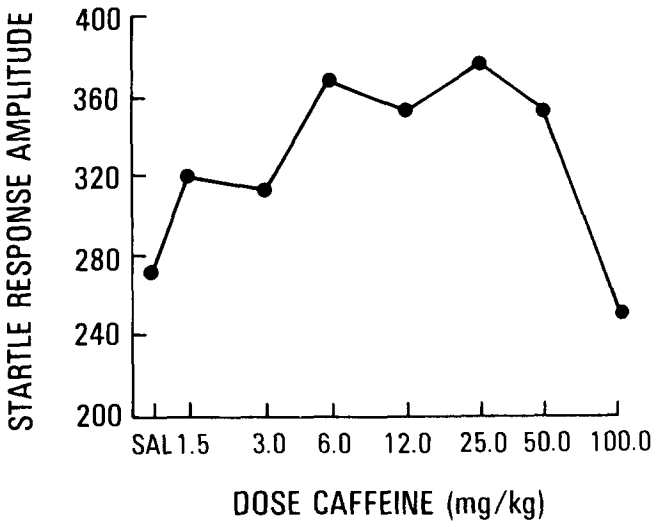


FIG. 1. The dose-response curve for the effect of caffeine (1.5 to 100 mg/kg, given subcutaneously 15 minutes prior to testing) on tactile startle in rats is shown as the group means (N=11+ per group) of the averaged response amplitudes across all 121 trials.

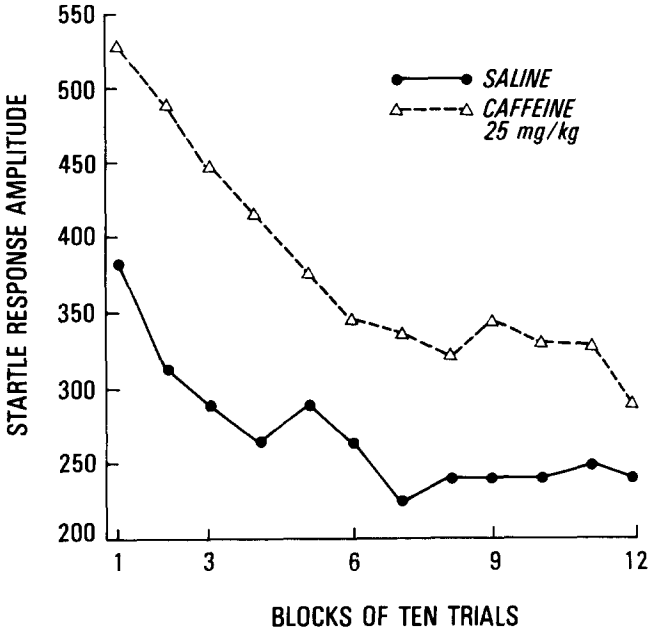


FIG. 2. The effect of 25 mg/kg caffeine on the startle responses of rats is shown as the group means (N=12 each group) for 12 successive blocks of ten responses each.

cabinet door was closed 5 minutes prior to the first stimulus. Each test consisted of 121 stimuli presented at 15 second intervals. The effects of various doses of caffeine (1.5 to 100 mg/kg) on tactile startle were examined in three separate experiments. To examine the time-course of caffeine's effect on startle, rats were injected with saline or 12 mg/kg caffeine and tested at 15 minute intervals after injection. Six tests of 11 trials each were given over a total of 90 minutes.

*Statistics*

The data from each test were reduced to the first response and 24 blocks of five responses each and transmitted over the phone to the University's central computer for analyses of variance (ANOVA) using BMDP2V [3]. For each experiment, a mixed-design ANOVA was done with drug treatment as a between-subjects factor and blocks of trials as a within-subjects factor. Differences in habituation trends were thus reflected in the treatment-by-trials interaction term.

## RESULTS

The results shown in Fig. 1 summarize the effects of various doses of caffeine on tactile startle averaged across all 121 trials. These results were compiled from three separate experiments. The largest study examined four doses of caffeine ranging from 6.0 to 50.0 mg/kg in addition to the saline-injected controls (N=11 or 12 per group). The resulting mixed-design ANOVA revealed a significant main effect of caffeine, $F(4,54)=2.66$, $p<0.05$, with no significant drug-by-trials interaction, $F(44,594)=1.07$, n.s. The effects of 25 mg/kg caffeine relative to controls are displayed in Fig. 2 as group means for the first response and 12 successive blocks of ten trials each across the 121 trial session. It can be seen in Fig. 2 that caffeine increased startle uniformly on all trials, rather than having a specific effect on habituation slopes.

The highest dose of caffeine tested, 100 mg/kg, did not affect startle uniformly on all trial blocks and the main effect of this dose was not significant, $F(1,23)=0.44$, n.s. Rather, 100 mg/kg caffeine increased startle on the first 20 trials and decreased it on subsequent trials, resulting in a significant treatment-by-trials interaction, $F(19,437)=2.78$, $p<0.001$. As a further indication of the dose-dependency of caffeine's effect, the lowest doses tested (1.5 and 3.0 mg/kg) were found to have no significant effect, $F(2,30)=1.56$, n.s., although they both produced mean increases in overall startle magnitudes (see Fig. 1).

In the study of the time-course of caffeine's effect on tactile startle, the maximal response was found at 15 minutes post-injection and was comparable in magnitude to that found for 12 mg/kg caffeine in the preceding dose-response study (Fig. 1).

## DISCUSSION

The results of these experiments demonstrate that caffeine significantly increases the magnitude of tactile startle responses in rats. Doses of caffeine from 1.5 to 50 mg/kg increased startle, while the highest dose tested, 100 mg/kg reduced startle, especially during the latter half of the test session. Thus, the effect of caffeine on startle is an inverted U-shaped function of dose, as is typical of many drug effects on measures of behavioral arousal. This pattern of effects due to caffeine is consistent with reports that arousal and memory performance in humans are augmented at low to medium doses of the drug and reduced at higher doses [4]. As illustrated in Fig. 2, the effect of caffeine on startle appears to reflect a general augmentation of behavioral reac-

tivity rather than a specific change in the inferred processes of habituation or sensitization [11]. That is, caffeine increased startle responses uniformly throughout the test session.

In the case of other psychostimulants such as amphetamine, the increased ambulation characteristic of low doses is replaced by stereotypic behaviors at higher doses [12]. It has been noted that caffeine is an unusual psychostimulant in that even high doses do not produce focussed stereotypy [5]. The present results suggest another, perhaps related difference between caffeine and amphetamine-like stimulants. In contrast to the effects of caffeine reported here, doses of amphetamine that produce marked increases in locomotor activity have no effect on either acoustic [1] or tactile startle [6]. The behavioral effects of caffeine and amphetamine upon startle reactivity and locomotor activity are therefore uncorrelated; a difference which suggests mechanistic differences between the two drugs.

It appears from these results that rat startle may provide a useful and unusually sensitive measure of the behavioral effects of caffeine. As a measure of drug effects in animals, startle has the unusual advantage of being amenable to very similar studies in man [8,9]. That similar effects would be expected to be seen in humans is suggested by a recent blind clinical trial of caffeine in prepubertal boys, designed to determine if, like amphetamine, caffeine may have therapeutic efficacy in the treatment of juvenile hyperkinesis [4]. In the course of demonstrating that caffeine dose not share with amphetamine the ability to calm such subjects, the trained observers did report that hyper-reactivity, or "jumpiness" was a prominent feature of the behavior of boys given three or ten mg/kg of caffeine [4]. "Fidgetiness" and hyperactivity were noted at the higher dose. If these phenomena are similar to our finding of enhanced startle in rats given caffeine, then hyper-reactivity should be demonstrable by measuring the blink reflex component of human startle. When testing parameters are similar, this measure of human startle appears to be comparable to measures of whole-body startle in rats, with regard to both habituation (Geyer and Braff, unpublished observations) and pre-pulse inhibition phenomena [9]. Therefore, preclinical testing of drugs in animals may be readily translated into comparable tests in humans by virtue of the similarity of startle phenomena across species. The importance of such an objective measure of caffeine's effects in man is evident from caffeine's widespread use and its reported association with anxiety, depression, increased hypnotic-sedative use, and clinical worsening of chronic schizophrenics [2,10].

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