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Effects of Nicotine, Caffeine, and Their Combination on Locomotor Activity in Rats

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COHEN, C., H. WELZL AND K. BÄTTIG. *Effects of nicotine, caffeine, and their combination on locomotor activity in rats.* PHARMACOL BIOCHEM BEHAV **40**(1) 121–123, 1991.—The interactive effect of caffeine and nicotine on spontaneous locomotor activity in a tunnel maze was determined in nicotine-naive and nicotine-tolerant rats. Rats were daily injected subcutaneously for 12 days with nicotine (0.4 mg/kg) to induce nicotine tolerance. Nicotine-naive rats were injected with saline. During the next two days, they were exposed to a tunnel maze for two 6-min trials. On the third day, locomotor activity was measured (30-min trial) in the tunnel maze 15 minutes after subcutaneous injection of saline, nicotine (0.2 mg/kg), caffeine (8 mg/kg), or nicotine (0.2 mg/kg) and caffeine (8 mg/kg) in combination. Acute exposure to nicotine decreased locomotor activity in nicotine-naive rats. This decrease was antagonized by simultaneous injection of caffeine. Chronic nicotine exposure induced the development of tolerance to the acute behavioral depressive effects of nicotine. In nicotine-tolerant rats, caffeine and nicotine in combination significantly increased locomotor activity above saline level, whereas given alone they had no significant stimulant effect. Neither chronic nicotine treatment nor acute drug treatments affected exploratory efficiency of rats.

Nicotine Caffeine Drug interactions Locomotor activity Tunnel maze Tolerance Rat

ALTHOUGH nicotine and caffeine are widely used stimulants, little is known about their interactive effect on behavior. Previous studies on the effect of caffeine on motor performance have generally reported that caffeine-induced stimulation is increased in situations involving fatigue, habituation or low motivational levels (3). During chronic caffeine intake a tolerance to the stimulant effect of caffeine on locomotor activity develops rapidly (1, 6, 9). When daily caffeine treatment is halted this tolerance quickly disappears within 48–72 h (9).

The effect of nicotine on motor performance has been extensively studied. The most consistent acute effect is a depression of activity. With repeated administration, however, tolerance develops to the depressant effect but not to a second, stimulant effect of nicotine (7, 8, 10, 14, 15). Thus, in nicotine-tolerant rats, nicotine stimulates locomotor activity. Nicotine tolerance persists for several weeks after termination of nicotine treatment.

To date, only few data exist on the combined effects of caffeine and nicotine on behavior in the rat (11,16). To investigate the interactive effect of caffeine and nicotine on locomotor activity, nicotine-naive and nicotine-tolerant rats were tested after nicotine and/or caffeine injections in the hexagonal tunnel maze. The tunnel maze allows the registration of different motor and cognitive variables continuously and automatically (2). This maze can be considered to be a natural and stimulating situation for the rat.

METHOD

Animals

Sixty-four male Wistar rats weighing 240 to 280 grams at the time of testing were used. Throughout the study, the rats were group-housed (4 per cage). Food pellets and tap water were available ad lib in the home cage.

Drugs

Nicotine hydrogen (+)-tartrate and anhydrous caffeine were dissolved in physiological saline. The dosages used were 0.2 and 0.4 mg/kg for nicotine (expressed as free base) and 8 mg/kg for caffeine. Doses of nicotine and caffeine were selected on the basis of earlier results (4,12). A systemic injection of 0.2 to 0.4 mg/kg nicotine in rats leads to plasma nicotine concentrations that are comparable to those observed in humans after smoking (5,13). During the pretreatment period, nicotine solutions were freshly prepared every third day. On the test day solutions were newly prepared.

Apparatus

The hexagonal tunnel maze consisted of an outer hexagonal ring, an inner hexagonal ring and an open field in the center, all connected through short radial arms. The diameter of the maze was 150 cm, each alley being 8 cm wide and 15 cm high. Forty-two infrared photocell units were distributed uniformly throughout the tunnel maze. They were connected via an interface to an IBM PC so that the location of a rat in the maze could be continuously monitored and recorded for later statistical evaluation. Repeated activations of the same photocell (e.g., AA) or a pair of photocells (e.g., ABAB) were not counted to avoid counting stereotyped behaviors in front of a photocell as activity. Walls, ceiling and all electronic photocell circuits of the maze system

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formed a compact unit which could be lifted from the floor to permit easy removal of the subject and subsequent cleaning of the floor. Each trial started with the rat in the open field.

Procedure

All testing was done between 0900 and 1300. The subjects were injected subcutaneously with saline or nicotine at a dose of 0.4 mg/kg in a total volume of 0.5 ml on 12 consecutive days (pretreatment: Days 1–12). On Days 13–14, the animals were exposed to the maze for a 6-min trial. On Day 15, the animals were injected intraperitoneally with saline, nicotine (0.2 mg/kg), caffeine (8 mg/kg), or nicotine (0.2 mg/kg) + caffeine (8 mg/kg), in a volume of 1 ml. The injected rat was then placed into a holding cage for 15 minutes before being placed into the tunnel maze system for a 30-min trial. At the end of the 30-min test session, the subject was removed and the floor wiped with a cloth dampened with a warm detergent solution to eliminate odor trails.

Behavioral Variables

Locomotor activity was represented by the total number of photocell interruptions per trial. Further, each 30-min trial (Day 15) was divided into five 6-min test intervals. The decline in locomotor activity over intervals provided a measure of intratrial habituation.

Exploratory efficiency was defined as the number of photocells visited until 36 different photocells (or 85% of the maze) were visited. A rat which frequently retraced alleys already visited and thus visited photocells two or more times, obtained a high score, reflecting low exploratory efficiency.

Statistical Analysis

The effect of chronic and acute drug treatments on locomotor activity were evaluated by performing a two-way (acute treatment × chronic treatment) between subject analysis of variance (ANOVA) with repeated measures (statistical program BMDP-2V). Since the acute treatment × interval interaction reached the level of significance (i.e., the rate of intrasession habituation differed between treatments) separate two-way analyses of variance were used for the 6-min test intervals (BMDP-7D). A two-way analysis of variance was also used for the total locomotor activity (BMDP-7D). In those cases where significant differences were found, Tukey's test was employed to determine which group means differed. The effect of chronic and acute drug treatments on exploratory efficiency was analyzed using a two-way between subject ANOVA (BMDP-7D).

RESULTS

Locomotor Activity

On Day 15, acute and chronic treatments significantly affected locomotor activity [acute treatment: $F(3,56)=5.79$, $p<0.01$, chronic treatment: $F(1,56)=20.17$, $p<0.001$, treatment interaction: $F(3,56)=10.21$, $p<0.001$]. During the 30-min trial on Day 15, mean locomotor activity decreased over the 6-min intervals in all groups; i.e., all rats showed an intratrial habituation [repeated measures: $F(4,224)=240.72$, $p<0.001$; Fig. 1]. The rate of habituation differed between acute drug treatments [acute treatment × repeated measures: $F(12,224)=6.29$, $p<0.001$], but chronic nicotine treatment did not affect habituation [chronic treatment × repeated measures: $F(4,224)=0.85$, n.s.].

An acute dose of nicotine (0.2 mg/kg) decreased total locomotor activity significantly ($p<0.01$) in nicotine-naive rats when compared with saline-injected controls (Table 1). This decrease was apparent during the first four test intervals (all p-values <0.05; Fig. 1, top panel). Total locomotor activity of saline-injected nicotine-tolerant rats did not differ from the activity of saline-injected nicotine-naive rats (Table 1). Further, in nicotine-tolerant rats, total locomotor activity was slightly increased by nicotine (0.2 mg/kg) from the second test interval on (Fig. 1, bottom panel), but this effect did not reach significance.

Caffeine given alone had no significant effect on total locomotor activity in nicotine-naive rats or in nicotine-tolerant rats (Table 1). It only slightly decreased locomotor activity during the first interval in both nicotine-naive and nicotine-tolerant groups (Fig. 1).

TABLE 1

EFFECT OF DRUG TREATMENTS ON TOTAL LOCOMOTOR ACTIVITY (TOTAL ACTIVITY COUNTS: MEAN ± SE)

Acute Treatment	Nicotine-Tolerant Groups	Nicotine-Naive Groups
Saline	564 ± 17	636 ± 22.3
Nicotine	652 ± 8.1	266 ± 24*
Caffeine	606 ± 18.3	573 ± 19
Nicotine + Caffeine	747 ± 16.9	520 ± 15.6

Data were analysed by a Tukey's test.
*$p<0.01$ compared with respective saline group.

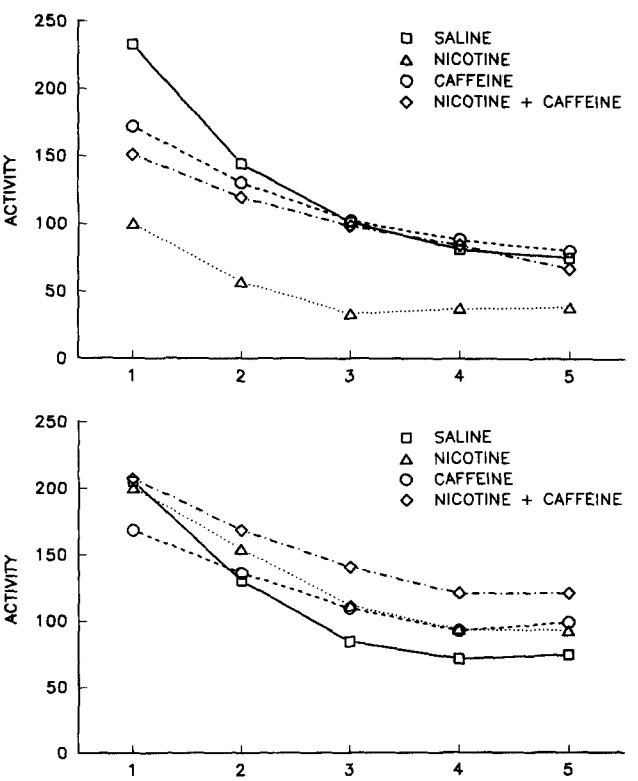

FIG. 1. Intrasession habituation for the successive 6-min time intervals are plotted for the 4 treatments (saline, nicotine 0.2 mg/kg, caffeine 8 mg/kg, nicotine 0.2 mg/kg + caffeine 8 mg/kg) in nicotine-naive rats (top panel) and in nicotine-tolerant rats (bottom panel).

Locomotor activity observed after a combined injection of caffeine plus nicotine reached the same activity level than that observed after an injection of caffeine alone. When the activity of nicotine-injected and nicotine plus caffeine-injected animals were compared, a significant difference in locomotor activity was obtained for the second, third, and fourth interval (all p-values <0.05; Fig. 1, top panel).

An injection of caffeine plus nicotine in nicotine-tolerant rats increased locomotor activity when compared with the respective saline-injected control group (Fig. 1, bottom panel). This increase reached significance during the third, fourth, and fifth interval (all p-values <0.05). Compared to saline-injected controls, the increase in locomotor activity after the combined injection was twice the increase in activity seen after an injection of nicotine or caffeine alone.

Exploratory Efficiency

The exploratory efficiency (total locomotor activity until 36 different photocells were visited) was not affected by chronic or acute drug treatments [chronic treatment: $F(1,56)=1.13$, n.s.; acute treatment: $F(3,56)=1.30$, n.s.; treatment interaction: $F(3,56)=0.98$, n.s.].

DISCUSSION

In the present experiment, an acute exposure to nicotine produced a decrease in locomotor activity. Depressed locomotor activity was observed during the entire 30-min trial. An acute exposure to caffeine did not significantly increase locomotor activity above the control level. According to a previous study from this laboratory (12), the absence of a stimulant effect of caffeine appears to be due to the elaborate maze arrangement rather than the dosage used.

Chronic nicotine exposure promoted the development of tolerance to the depressant effect of nicotine but was not found to affect significantly the response to caffeine. A minor stimulant effect of caffeine, however, was visible in nicotine-treated rats.

Finally, the present results suggest that caffeine regulates the effects of nicotine on locomotor activity in nicotine-naive rats and, to a lesser extent, in nicotine-tolerant rats. In nicotine-naive rats, the interaction of the effects of nicotine and caffeine resulted in an abolition of the locomotor depressant effects of nicotine by caffeine. In nicotine-tolerant rats, nicotine and caffeine administered together was found to increase locomotor activity above the control levels whereas, given alone, a small but not significant increase in locomotor activity could be seen.

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