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# COMMUNICATIONS

## Effect of caffeine and nicotine on avoidance learning in mice: lack of interaction

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**Abstract**—Tested alone, nicotine (0·25 or 0·5 mg kg$^{-1}$) improved shuttle-box avoidance learning in mice of the CD-1 strain. Caffeine had no effect at doses of 2·5 and 5 mg kg$^{-1}$ and impaired performance at a dose of 10 mg kg$^{-1}$. Combinations of the two drugs did not increase avoidance responses more than nicotine alone, nor was nicotine able to attenuate performance depression induced by the highest dose of caffeine. Lack of drug interaction in the avoidance test contrasts with the occurrence of interactive effects of the two drugs in a locomotor activity test. When given in combination, caffeine and nicotine increased locomotor activity at doses ineffective by themselves. The results seem to indicate no advantage in combining caffeine and nicotine to improve active avoidance learning.

In spite of the wide use of caffeine and nicotine as psychoactive agents, there are few reports on the behavioural effects of the two drugs combined. The type of interaction between caffeine and nicotine varied depending on drug doses and experimental conditions. Lee et al (1987) observed that both caffeine (6 mg kg$^{-1}$) and nicotine (0·5 mg kg$^{-1}$) enhanced locomotor activity in rats, when given alone, and exerted additive stimulating effects, when combined. In another activity test, nicotine reduced locomotion at a dose of 0·2 mg kg$^{-1}$ in drug-naive rats previously exposed to the testing environment; such a depressant action of nicotine was antagonized by 8 mg kg$^{-1}$ caffeine, a dose ineffective by itself (Cohen et al 1991). In a schedule-controlled operant behaviour study, the rate-increasing action of nicotine (0·3 mg kg$^{-1}$) was enhanced, in an additive manner, by a stimulatory dose of caffeine (3 mg kg$^{-1}$), but was antagonized by higher doses. In the present research, the investigation of possible interactive effects of the two drugs was extended to a learning task. Learning ability was tested in mice subjected to shuttle-box avoidance training. Combinations of caffeine and nicotine were also tested for their effects on locomotor activity in mice.

Previous animal studies indicated that, depending on species, strain, nature of task and dosage, caffeine (Nehlig et al 1992) and nicotine (Levin 1992) may improve or impair learning and memory, or have no effect. Facilitating effects on active avoidance acquisition were reported for nicotine (Levin 1992) more often than for caffeine (Nehlig et al 1992).

### Materials and methods

*Animals.* The subjects were naive male mice, 8–9 weeks old, of the randomly bred CD-1 strain (Charles River, Italy). Upon their arrival in the laboratory (7–10 days before the experiment) the mice were housed in standard transparent plastic cages (eight per cage) under standard animal room conditions (free access to food and water, 12 h light/dark cycle, ambient temperature of 23°C). The experiments were carried out between 0900 and 1600 h using different animals for different tests. Each experimental group included eight mice.

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*Drugs.* Saline (0·9% NaCl), caffeine (anhydrous powder; 2·5, 5 or 10 mg kg$^{-1}$) and nicotine bitartrate (0·25 or 0·5 mg kg$^{-1}$, doses expressed as free base), dissolved in distilled water, were injected intraperitoneally in a volume of 10 mL kg$^{-1}$. The pH of the nicotine solutions was adjusted to 7 with NaOH. Combinations of drugs were given as mixed solutions, in a single injection.

*Active avoidance.* The apparatus consisted of eight automated shuttle-boxes, each divided into two 20 × 10 cm compartments, connected by a 3 × 3 cm opening. A light (10 W) was switched on alternately in the two compartments and used as a conditioned stimulus (CS). The CS preceded the onset of the unconditioned stimulus (US) by 5 s and overlapped it for 25 s. The US was an electric shock (0·2 mA) applied continuously to the grid floor. The inter-trial interval was 30 s. An avoidance response was recorded when the animal avoided the US by running into the dark compartment within 5 s after the onset of the CS. If animals failed to avoid the shock they could escape it by crossing during the US. Failure of both avoidance and escape responses in one trial resulted in the loss of the following trial for an individual mouse. In this case the percentage of avoidance responses was calculated on the actual number of trials to which the mouse was subjected. However, failure to escape seldom occurred in the present experiment. Spontaneous crossings from the dark to the light compartment were punished and recorded as inter-trial responses.

Training consisted of five daily 100-trial sessions. Fifteen minutes before each avoidance session, the mice received caffeine or nicotine, alone or combined. Doses of the two drugs were chosen on the basis of previous studies (Sansone 1975; Sansone et al 1991a), carried out with mice of the CD-1 strain. Control animals received saline only.

*Locomotor activity.* As in previous studies (Sansone et al 1991a, b), spontaneous locomotor activity was measured using the same apparatus employed to measure active avoidance. For this purpose the lamps of the shuttle-boxes were switched off and no electric shock was applied to the floor. For each mouse, the number of crossings from one compartment to the other was recorded for 30 min. Drug treatment consisted of the administration of saline, caffeine or nicotine, alone or combined, 15 min before testing. Nicotine was tested at a dose (0·5 mg kg$^{-1}$) that in a previous study (Sansone et al 1991a) improved shuttle-box avoidance learning in mice of the CD-1 strain, without affecting locomotor activity. Mice were used only once.

*Statistical analysis.* Drug effects on locomotor activity were evaluated by a two-factor analysis of variance, the factors being caffeine (four levels) and nicotine (two levels). Avoidance responses were evaluated by a three-factor analysis of variance, because in addition to the above two factors, caffeine (four levels) and nicotine (three levels), a third factor (repeated measures) was represented by daily sessions (five levels). Post-hoc analysis was carried out, when appropriate, by Duncan's multiple-range test.

## Results

*Active avoidance.* Fig. 1 reports, for all the experimental groups, the mean percent avoidance responses in each daily session and in the five sessions combined.

A three-factor analysis of variance for avoidance responses showed significant main effects of caffeine ($F(3,84):11{\cdot}05$, $P < 0{\cdot}001$), and nicotine ($F(2,84):3{\cdot}14$, $P < 0.05$), but no significant drug interaction ($F(6,84):0{\cdot}65$, $P > 0{\cdot}05$) on the whole of the five training sessions. The analysis also showed a significant effect of training ($F(4,336):112{\cdot}74$, $P < 0{\cdot}001$) and significant interactions caffeine × sessions ($F(12,336):7{\cdot}10$, $P < 0{\cdot}001$) and nicotine × sessions ($F(8,336):2{\cdot}45$, $P < 0{\cdot}05$). The three-factor interaction term was not significant. Duncan's test, for the five sessions combined, indicated no significant effect of caffeine, at doses of 2·5 and 5 mg kg$^{-1}$, while the highest dose (10 mg kg$^{-1}$) significantly reduced avoidance responses. Nicotine improved avoidance performance, but the effect was significant only at the dose of 0·25 mg kg$^{-1}$. When the two drugs were combined, caffeine did not enhance the improving action of nicotine on avoidance acquisition. Moreover, the performance impairment induced by the highest dose of caffeine was not antagonized by nicotine.

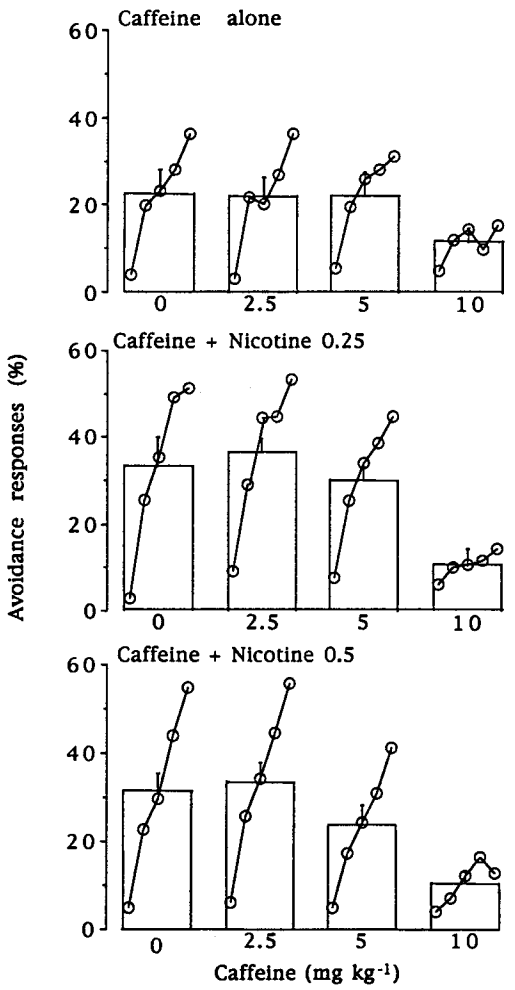


FIG. 1. Effect of caffeine and nicotine on shuttle-box avoidance acquisition in mice. Mean percent avoidance responses on the whole of the five 100-trial daily sessions (columns) and in each session (circles), in groups of eight mice. Vertical lines indicate s.e.m. Caffeine, at the doses of 0, 2·5, 5 or 10 mg kg$^{-1}$, and nicotine, at the doses of 0 (caffeine alone), 0·25 or 0·5 mg kg$^{-1}$, were injected intraperitoneally, alone or combined, 15 min before each daily session. Control mice (dose 0 of both drugs) received saline.

Table 1. Effect of caffeine alone or combined with nicotine (0·5 mg kg$^{-1}$) on locomotor activity in mice.

| Caffeine (mg kg$^{-1}$) | Locomotor activity (mean number of crossings, ± s.e., in 30 min) | |
|---|---|---|
| | Without nicotine | With nicotine |
| 0 | 85·7 ± 4·8 | 72·7 ± 10·6 |
| 2·5 | 85·0 ± 9·8 | 79·5 ± 9·2 |
| 5 | 88·5 ± 13·0 | 108·0 ± 10·6† |
| 10 | 87·7 ± 8·7 | 137·5 ± 12·6* |

Control mice (dose 0 of both drugs) received saline. *$P < 0{\cdot}01$ in comparison with saline, nicotine and corresponding dose of caffeine alone; †$P < 0{\cdot}05$ in comparison with nicotine alone.

No significant difference among groups was found for inter-trial responses. These responses, punished by electric shock, were present at the beginning of training (2–6%) but gradually disappeared as training proceeded.

*Locomotor activity.* Table 1 reports the mean activity crossings exhibited, in a 30-min session, by mice receiving caffeine (0, 2·5, 5 or 10 mg kg$^{-1}$) and nicotine (0 or 0·5 mg kg$^{-1}$), alone or in combination. A two-factor analysis of variance showed a significant main effect of caffeine ($F(3,56):3{\cdot}42$, $P < 0{\cdot}05$), but not of nicotine ($F(1,56):0{\cdot}14$, $P > 0{\cdot}05$), and a significant drug interaction ($F(3,56):5{\cdot}19$, $P < 0{\cdot}01$). A post-hoc analysis indicated that the two drugs, given alone, did not affect locomotor activity, while combinations of caffeine (5 and 10 mg kg$^{-1}$) and nicotine produced significant stimulating effects.

## Discussion

In agreement with previous studies (Bovet et al 1966; Barlow et al 1970; Sansone et al 1991a), in the present research nicotine improved shuttle-box avoidance acquisition in mice. Caffeine did not affect avoidance acquisition at the lowest doses used, but impaired performance at the highest dose. Previous studies reported that, in active avoidance tests, the effects of caffeine depended on animals and experimental procedures (Nehlig et al 1992). The present findings demonstrate no advantage in combining caffeine and nicotine to improve avoidance learning; caffeine did not enhance the improving action of nicotine and the facilitating effect of nicotine was blocked by caffeine at the highest dose. The latter effect is in agreement with the antagonism exerted by high doses of caffeine against the rate-increasing action of nicotine on operant behaviour of rats (White 1988). While no interaction between caffeine and nicotine occurred during avoidance training, the two drugs showed interactive effects on locomotor activity. Combinations of caffeine and nicotine produced locomotor-stimulating effects in mice, never observed with drugs given separately. In a previous study, doses of the two drugs, stimulating locomotor activity even if given alone, exerted additive stimulatory effects when combined (Lee et al 1987). As a consequence of the interactive effects occurring in the present study, a mixture of caffeine and nicotine ($10 + 0{\cdot}5$ mg kg$^{-1}$) that impaired avoidance performance, strongly stimulated locomotor activity.

The main mechanism of action of caffeine is antagonism at the level of adenosine receptors (Nehlig et al 1992), while nicotine mainly activates nicotinic acetylcholine receptors (Wonnacott 1990), but many behavioural effects of the two drugs seem due to interference with monoaminergic neurotransmitter systems (Fuxe et al 1990; Mitchell et al 1992; Nehlig et al 1992). In

some instances, as in the case of visual tracking behaviour, both caffeine and nicotine exerted effects similar to those of dopamine agonists (Evenden et al 1993). In other cases, similar behavioural effects of the two drugs, such as locomotor stimulation, were ascribed to different mechanisms of action (Lee et al 1987). Also in the case of avoidance behaviour, it seems probable that the two drugs act through different mechanisms, as previous and present findings indicate that active avoidance improvements may be obtained with nicotine more than with caffeine. It may be supposed that catecholaminergic, particularly dopaminergic mechanisms are responsible for the avoidance-facilitating effects of nicotine, in view of the role played by these mechanisms in acquisition and maintenance of aversive learning (Oei & King 1980), as well as in various behavioural and cognitive effects of the drug (Levin 1992). On the other hand, the effects of caffeine on dopamine are not clear and only a few cases of avoidance facilitation by this drug have been reported (Nehlig et al 1992). It is difficult to explain, on a neurochemical basis, the discrepancy between the interactive effects of caffeine and nicotine on locomotor activity and the lack of drug interaction on active avoidance performance.

To explain the different behavioural effects produced by caffeine-nicotine combinations, it should be considered that mice tested for locomotor activity received a single drug injection, while drug effects on avoidance learning were assessed by administering the drugs before each of five daily sessions. Repeated exposure to nicotine may increase the behavioural stimulant effect of the drug (Ksir et al 1987), while tolerance to the locomotor-stimulating action of caffeine develops quickly (Holtzman & Finn 1988). Behavioural depression during the first days of chronic caffeine ingestion has also been observed (Nikodijevic et al 1993). Thus, the repeated administration of caffeine and nicotine during avoidance training, might have played an important role in the effects on learning of the two drugs, alone or combined. In this respect, it must be noted that, in the present experimental situation, learning and its facilitation by nicotine developed gradually during the five-day training.

In view of the wide social use of coffee and tobacco and of the psychoactive properties of their components caffeine and nicotine, it seems important to ascertain the occurrence of interactive effects of these two drugs on cognitive processes. The present study, representing a step in this direction, indicates that caffeine does not enhance the improving action of nicotine on shuttle-box avoidance acquisition in mice. However, it cannot be excluded that the two drugs may exert interactive effects in other learning tasks.

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