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Nicotine Induced Locomotor Activity in Rats: The Role of Pavlovian Conditioning

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HAKAN, R. L. AND C. J. KSIR. *Nicotine induced locomotor activity in rats: The role of Pavlovian conditioning.* PHARMACOL BIOCHEM BEHAV **29**(4) 661–665, 1988.—Rats repeatedly exposed to small doses of nicotine will demonstrate a significant augmentation of locomotor activity in response to a subsequent test dose of nicotine. A sensitization of brain tissue is hypothesized to account for this effect but Pavlovian conditioning might also be a major factor. Therefore the present study assessed the possible role of Pavlovian conditioning in this nicotine effect. Two experiments were conducted. In the first, subjects were administered either saline or nicotine in either their home cages or in activity test cages for five days. All subjects were then tested in the activity test cages on day six. In the second experiment rats were administered either nicotine or saline in the presence of a complex stimulus and later tested for response to nicotine alone and the complex stimulus alone. Results from these experiments indicate that Pavlovian conditioning does not play a major role in nicotine's effect on locomotor activity.

Nicotine Locomotor activity Receptor regulation Pavlovian conditioning

REPEATED exposure to low doses of nicotine has been reported to produce an augmented stimulatory effect on locomotor activity in rats [3, 7, 12]. While there are several possible explanations for this phenomenon, it has recently been shown that rats given single daily low dose injections of nicotine for five consecutive days show an elevation in number of central nicotinic cholinergic receptors. This increase of receptor number appears causally related to a concomitant nicotine induced augmentation of locomotor responsiveness which is also centrally mediated [5]. Nicotine induced elevations of central nicotinic receptors have also been reported elsewhere [6,9]. On this basis we have proposed that the augmentation of locomotor activity in response to repeated nicotine exposure is due to a biochemical sensitization of brain tissue [5].

Another factor which might influence the behavioral response to repeated drug administration is Pavlovian conditioning [2,11]. In this context it is assumed that the drug acts as an unconditioned stimulus and stimuli which are present at the same time as the drug (e.g., stimuli associated with the injection procedure, test environment, etc.) may come to act as conditioned stimuli. These conditioned stimuli in turn may produce conditioned responses which alter the unconditioned drug effects. For example, it has been shown that morphine analgesia tolerance is greatest when animals are tested in the same environment in which tolerance development is attained. This effect of environment on tolerance is attenuated by Pavlovian extinction trial preexposure to the test environment, thus demonstrating that drug effects can be modified by Pavlovian learning processes [10,11]. It is important to remember that drugs may produce a variety of unconditioned responses in an animal, some of which may relate to compensatory reactions to the drug and it is assumed that compensatory reactions tend to counteract some of the direct effects of the drug (tolerance). Significant aspects of morphine analgesia tolerance are apparently due to conditioned compensatory responses to morphine which builds up with repeated administrations [10,11].

While conditioning factors have predominantly been documented in regard to decreased drug responsiveness, there have been at least some reports of conditioning phenomena associated with increased drug responsiveness. In this case it is assumed that conditioned stimuli elicit responses in the same direction as the unconditioned drug effects so that with repeated administrations conditioned responses add to the direct actions of the drug. For instance, cocaine induced stereotypy sensitization is most pronounced when cocaine is administered in the presence of distinctive cues. If the animals are then given extinction trials in which saline is administered in the presence of the previously drug-paired cues, the sensitization observed on a subsequent drug trial is attenuated [4]. A similar type of effect has been reported in association with cocaine induced hyperactivity. Rats which were administered cocaine in test cages became increasingly responsive to the drug, while rats administered cocaine in their home cages and saline in test cages showed little sensitization when subsequently challenged in the test cages with cocaine [8]. Therefore one possible explanation for our observation that repeated exposure to nicotine in rats produces an augmentation of locomotor responsiveness is that stimulatory actions of nicotine are enhanced with repeated exposures by a conditioned response which is in the same direction as the unconditioned response.

In a previous experiment [5], we gave rats daily 0.2 mg/kg

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TABLE 1

TEST CAGE AND HOME CAGE TREATMENTS FOR THE DIFFERENT GROUPS IN EXPERIMENT 1 (SEE TEXT FOR DIFFERENT GROUP CODES)

Group	Test Cage Treatment	Home Cage Treatment
NT-SH	0.2 mg/kg nicotine	saline
ST-SH	saline	saline
ST-NH	saline	0.2 mg/kg nicotine
SH-T	no injections	saline
NT	0.2 mg/kg nicotine	no injections
ST	saline	no injections
NH	no test cage exposures	0.2 mg/kg nicotine
SH	no test cage exposures	saline

Home cage treatments occurred 4 1/2 hr after test cage treatments.

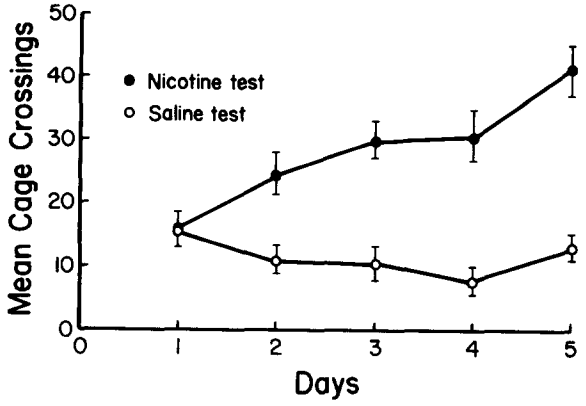

FIG. 1. Mean cage crossings in the first ten minutes following daily injections of saline or 0.2 mg/kg nicotine.

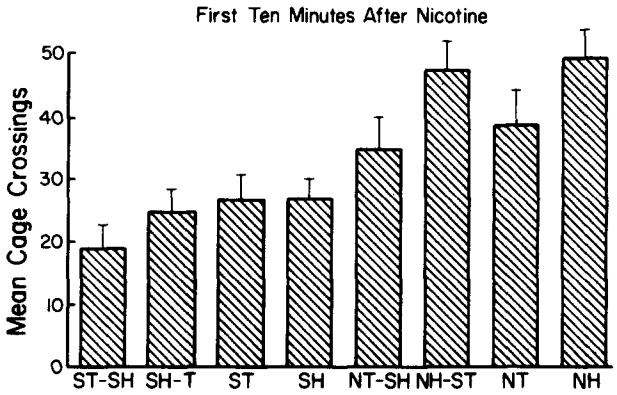

FIG. 2. Mean cage crossings for each group following 0.2 mg/kg nicotine injections on the test day (day six).

nicotine injections in their home cages for five days. The only distinctive stimuli predictive of nicotine for these rats were the stimuli related to being removed from the home cages, given an injection and then returned to their home cages. Some of these rats were then given saline injections in their home cages once/day for 7 days. These would constitute extinction trials in the classical conditioning paradigm, since injection-related stimuli no longer predicted nicotine. Following 7 days of extinction trial procedures these animals were given a test dose of nicotine in activity test cages. The test day locomotor response to nicotine in these animals was significantly greater than that seen in control animals. A subsequent binding assay showed that central cholinergic nicotinic receptor populations were also significantly elevated as compared to controls. From these results it appears that the augmentation of locomotor stimulation produced by repeated exposure to nicotine is more probably caused by the increased number of nicotinic receptors than by classical conditioning. Nevertheless, conditioning might certainly interact with nicotine action in such a way as to enhance or diminish its effects. The current study was designed to assess more explicitly whether conditioning phenomena play a role in the augmentation of activity which is seen in rats after five days of nicotine exposure.

METHOD

Subjects

Forty-eight adult male Holtzman-derived rats were used (n=6/group). Subjects were housed in groups and had free access to food and water.

Apparatus-Activity Test Cages

Activity was measured in 48×19×19 cm test cages that monitored locomotion as sequential breaks of two infra-red photocell beams which emanated from the side walls of the chamber, 14 cm from either end. Interruption of one cell followed by interruption of the other cell was counted as a single crossing. Cumulative crossings during ten minute intervals were monitored and recorded via a microcomputer printer system.

PROCEDURES

Experiment 1: Place Conditioning

All rats were given daily injections for five days and were tested on the sixth day. Some of the rats were given injections of nicotine (0.2 mg/kg, SC, calculated as nicotine base) on the exposure days and some of the rats were given saline. Injections were given either while the rats were in their home cages or one hour after they had been transferred to the test cages, where they remained for another hour after the injection. Some groups received only a single daily exposure injection of either nicotine or saline while other groups received injections of both saline and nicotine; the first in the test cages and the second two hours after being returned to their home cages. Table 1 describes the injections given each of the eight groups on exposure days 1–5.

Following the exposure days described above, on day six all subjects were administered a 0.2 mg/kg test dose of nicotine in the activity test cages. Again, all subjects were allowed a one hour adaptation period prior to injection.

RESULTS

The predominant response to nicotine was seen in the first ten minutes following injection, consequently all results refer to the effect observed in this time period unless otherwise specified. Figure 1 shows activity following test cage injections for nicotine and saline groups (NT, NT-SH, ST, and ST-SH) from day one to day five. Relative to saline group responding a locomotor sensitization occurred in the nicotine group which was similar to sensitized responses we

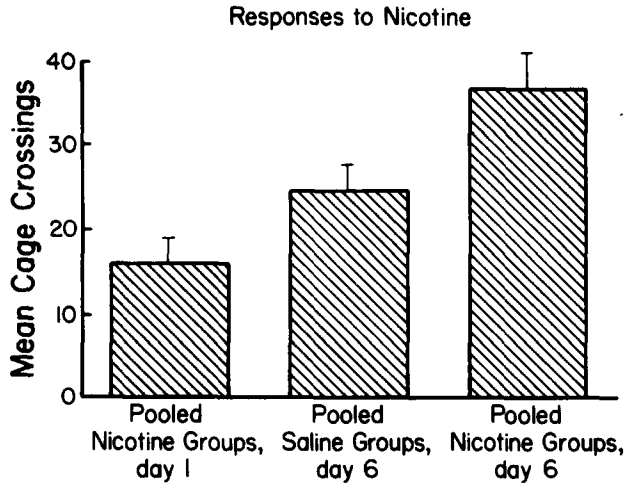

FIG. 3. Test day response to nicotine in groups pretreated with saline or nicotine compared to the day one response in the nicotine pretreated groups.

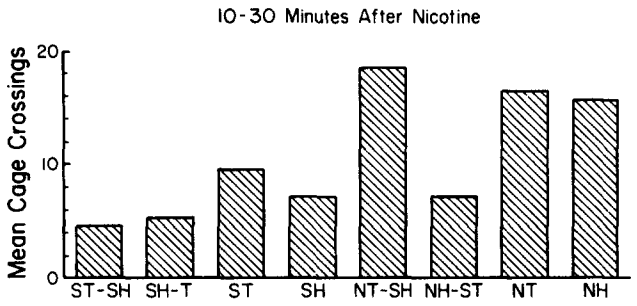

FIG. 5. Mean cage crossings for each group occurring ten to thirty minutes after test day injection.

have observed in the past [5]. A mixed-design ANOVA on crossings during the first ten minutes found the difference between saline and nicotine groups to be highly significant, $F(1,21)=26.5$, $p<0.001$, as was the effect of days, $F(4,84)=5.6$, $p<0.001$, and the group by days interaction, $F(4,84)=8.5$, $p<0.001$.

Figure 2 shows activity in the first ten minutes on day six after all animals received the test dose of nicotine. A three-way ANOVA for nicotine vs. saline, home cage administration of nicotine vs. test cage administration of nicotine and for test cage pretest exposure vs. no preexposure (extinction trial vs. no extinction trials) indicated that the effect of nicotine was significant, $F(1,38)=18.9$, $p<0.001$, but neither the effect of home cage vs. test cage nicotine administration nor the effect of pretest exposure vs. no pretest exposure to the test apparatus were significant. The interactions of these factors were also nonsignificant.

The two groups previously given nicotine in their home cages appeared to show a greater test day response to nicotine than the groups previously exposed to nicotine in the test cage environment. However, combining groups NH-ST with NH and comparing them with the combined NT-SH and NT groups showed that this trend was not significant, $t(21)=1.86$, $p=0.076$.

The test day response to nicotine in saline treated subjects was relatively high compared to the magnitude of response typically associated with day one nicotine exposure. Figure 3 shows the mean test day nicotine response for the

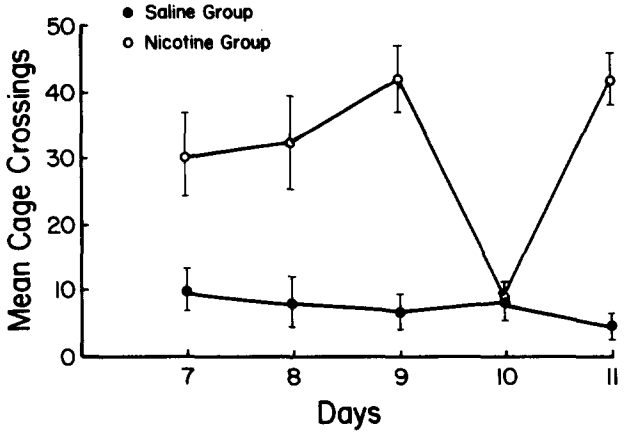

FIG. 4. Mean pooled responses to nicotine on the test day (day six) for nicotine pretreated groups and saline pretreated groups over the fifty minutes following injection. P: mean cage crossings in the ten minute interval preceding injection.

first ten minutes for groups previously treated with saline injections (ST, ST-SH, SH, and SH-T, 24.6) compared to the mean ten minute response on day one in subjects administered nicotine in the test cages (NT, and NT-SH, 15.83). A t-test analysis indicated a significant difference, $t(32)=2.53$, $p=0.01$.

While the predominant effect of nicotine was seen in the first ten minutes after injection on the test day, a long term response in the nicotine treated groups is also evident in Fig. 4. Unfortunately data from periods four and five in the NH group were lost and consequently we analyzed this long term response only in the twenty and thirty minute time periods. Figure 5 shows the mean response at twenty and thirty minutes in all groups. A three-way ANOVA for nicotine vs. saline, home cage administration vs. test cage administration and extinction trials vs. no extinction trials indicated that the long term response in the nicotine treated groups was significantly different from the saline treated groups, $F(1,39)=16.1$, $p<0.001$. No other main effect was significant. However, the drug × administration environment interaction was significant, $F(1,39)=4.19$, $p<0.05$. This interaction reflects the fact that the NH group did not significantly differ in long term response from groups that received nicotine in the test cages, while the long term response of the NH-ST group was significantly diminished, $t(12)=2.19$, $p<0.04$.

Experiment 2: Signalled Drug Administration

Sixteen animals were given daily test cage sessions in a darkened room. Following the adaptation period in these daily sessions, a complex visual and auditory stimulus was initiated one minute before eight animals each were injected with either 0.2 mg/kg nicotine or saline. The stimulus consisted of a strobe light and a tape recorded complex auditory stimulus (music). The strobe light was located approximately two meters from the test cages and emitted approximately 250 footcandles at its source. The auditory stimulus produced an average output of 72 dB in a background noise level of 64 dB. This unique stimulus was then allowed to continue throughout the following one hour monitoring period. This procedure was followed for nine days. Day ten procedures were the same except that the nicotine group received saline instead of nicotine. On day eleven saline was

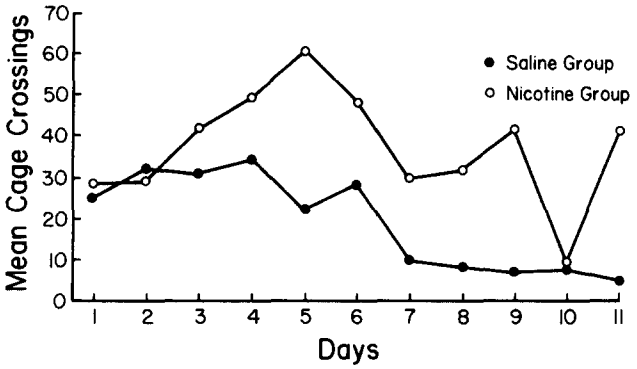

FIG. 6. Mean cage crossings during the first ten minutes after injection on days one through eleven for groups given saline or nicotine. On days one through nine saline or nicotine was given in the presence of a complex stimulus. On day ten both groups received saline in the presence of the previously conditioned complex stimulus. On day eleven the nicotine group was again given nicotine but in the absence of the complex stimulus.

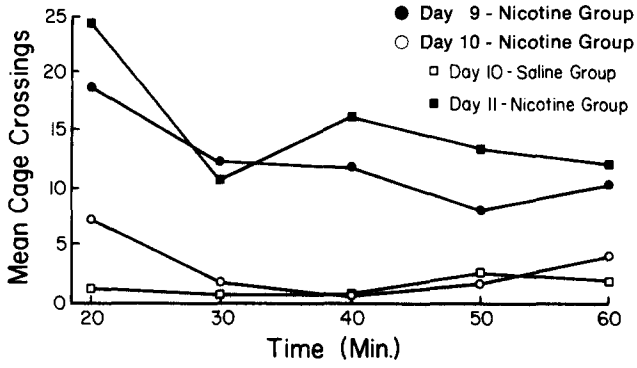

FIG. 7. Mean cage crossings for the saline and nicotine groups on days nine, ten and eleven for time periods 20–60 minutes. On day nine, groups received saline or nicotine in the presence of a complex stimulus. On day ten, both groups received saline in the presence of the complex stimulus while on day eleven, groups received either saline or nicotine but in the absence of the complex stimulus.

administered to the saline group and nicotine was again administered to the nicotine group but in the absence of the unique stimulus.

RESULTS

Signalled Drug Administration

Results from the signalled drug administration study are demonstrated in Fig. 6. A fairly typical behavioral augmentation is seen from days one to five in the nicotine treated group, and is maintained through day nine. The day ten signalled saline response for the nicotine group in the first ten minutes after injection was significantly attenuated relative to the nicotine response observed on day nine, $t(14)=6.02$, $p<0.0003$, and not significantly different from the day ten response seen for the saline treated group, $t(14)=0.34$, $p<0.7$. Response in the first ten minutes following unsignalled nicotine administration on day eleven is significantly elevated from day ten saline response in the nicotine group, $t(14)=7.09$, $p<0.00005$, and not significantly different from the signalled nicotine response seen on day nine, $t(14)=.019$, $p<0.98$. Figure 7 compares the long term responding observed in the saline and nicotine group on signalled drug day nine to long term responding on days ten and eleven. A mixed-design ANOVA found long term responding in the nicotine group on day nine to be significantly greater than day ten, $F(1,14)=8.3$, $p<0.025$. Long term response on day eleven was also significantly greater than that seen on day ten, $F(1,14)=7.3$, $p<0.025$, but not significantly different from day nine responding, $F(1,14)=0.28$, $p<0.5$.

DISCUSSION

The behavioral sensitization seen in these experiments conforms well to that seen in previous studies [5]. Since the nicotine response in the first ten minutes of a test session is not significantly influenced by the environmental context in which previous injections were given, the most parsimonious interpretation of these results is that the increased effect of nicotine on locomotor behavior has a predominantly biochemical basis. Moreover, if one were to speculate about the trends seen in this study, biochemical predominance of the nicotine response would apparently be even more substantiated since the animals administered nicotine in their home cages subsequently tended to respond more than the test cage nicotine animals when tested in the activity cages.

The only significant difference seen on the test day among the groups treated with nicotine under different conditions occurred in the later time periods. The NT-SH, NT and NH groups all showed responding in the later periods which was greater than that seen in the saline treated groups. These groups were also significantly more active than the NH-ST group which implies that the later response is in some fashion influenced more by procedural variables than by the pharmacological actions of nicotine alone. A conditioning explanation of this difference can be formulated; it is possible that the NT, NT-SH and NH groups have come to associate drug administration or drug administration and environment with the pharmacological action of nicotine and then conditionally respond in the later periods following the dissipation of a direct action of nicotine on locomotor activity. This explanation is in agreement with evidence from nicotine discrimination studies which demonstrate that the nicotine cue is available to rats for up to 80 minutes after administration (Hirschhorn and Rosecrans, 1974). The NH-ST group is procedurally different from the other nicotine groups only in that they were given extinction trials in the test apparatus on days one through five before being tested on day six. If this procedural difference associated the test cages with "safety" in the NH-ST group then test day response in the later periods may have been attenuated due to counter conditioning. However, the results of our second experiment failed to demonstrate a conditioning effect in either the first ten minutes following injection or in the later time periods, therefore we feel confident that Pavlovian conditioning is not a significant factor in nicotine response.

Tangentially, it appears that the process of simply handling and injecting subjects in some way enhances the subsequent effect of nicotine since animals treated with saline during the first five days responded on the test day at a significantly higher level than that observed on day one in animals treated with nicotine. This was essentially true regardless of other procedural variables. No strong argument can be made here in explanation of this phenomenon. It is worth suggesting however that another facet of learning may be involved. The handling and injecting of naive rats is probably an aver-

sive event for them and might reasonably be expected to produce an emotional response. On the other hand, repeated episodes of handling and injection could easily habituate the rats to these aversive procedures. The idea that habituation occurs to the effects of handling and injection is generally supportable from the observation that the locomotor response in saline treated animals is highest on day one in both the ST-SH group and the ST group. It is possible that an habituation process is important in the manifestation of a behavioral response to nicotine. If saline pretreated animals respond to test day nicotine in greater magnitude because they have been habituated to the emotion arousing aspects of the injection procedure then the same process is likely to occur in the nicotine treated animals. Thus on the first few days of nicotine administration little habituation has occurred and locomotor activity is less influenced by nicotine while by day five, animals have in large habituated to injection procedures and consequently a greater effect of nicotine can be observed on locomotor activity. But again, the increase of nicotine response due to habituation is probably far outweighed by the direct action of nicotine on central motor systems since test day response in nicotine treated animals is much greater than that seen in saline treated animals.

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