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Timing of conditioned responding in a nicotine locomotor conditioning preparation: Manipulations of the temporal arrangement between context cues and drug administration

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Abstract: Using a locomotor conditioning preparation, we examined whether manipulating time between exposure to distinct environmental cues and nicotine administration affected conditioned responding. Rats that received nicotine (0.42 mg/kg base) immediately before placement in an environment for 30 min on eight separate occasions displayed hyperactivity relative to controls in a subsequent injection/drug-free test. This conditioned hyperactivity was weaker if nicotine was administered 15 min before environment exposure. Conditioning was not evidenced when nicotine was administered 15 min after placement or upon removal from the environment. In a follow-up experiment, rats received 45 min in the environment; nicotine was administered 15 min after placement. This group showed conditioning that was localized to the last two-thirds of a 45 min test indicating that a 15 min delay did not prevent conditioning given 30 min of environment/nicotine overlap. This apparent timing of conditioned responding was not due to increasing environment exposure to 45 min. Further, a state-dependent environmental familiarization account of locomotor hyperactivity during testing was eliminated by the finding that rats displayed temporally specific increases in activity on the test day despite the fact that the context was previously experienced without drug for 15 min on eight consecutive days.

Keywords: Pavlovian conditioning, Interstimulus interval, Nicotinic, Sensitization, Smoking, Temporal learning, Tobacco

1. Introduction

In part, Pavlovian (classical) conditioning is thought to be involved in processes mediating drug abuse. These processes may include cue-evoked withdrawal and/or cravings (urges) often used to explain the maintenance of habitual drug use and relapse after abstinence [21, 27, 34, 36, 40]. Similarly, scientific inquiry into nicotine and tobacco use implicates Pavlovian conditioning processes [20, 23, 35]. For instance, Lazev et al. [23] repeatedly paired a complex polymodal stimulus (termed conditioned stimulus or CS) with access to smoking a cigarette (designated unconditioned stimulus or US) in young adults. Across repeated pairings of the CS and US, subjects' pulse rate and Likert-scale reports of urges increased during the CS. This change in response did not occur

to a second stimulus that was never paired with cigarette access. This differential control of urges and pulse was taken as evidence for a conditioned association between the polymodal CS and the appetitive effects of nicotine.

These nicotine-conditioned associations can be studied using various preclinical models [10, 18, 33, 43]. Recently, our laboratory has employed a locomotor conditioning task with rats ([5, 7, 30, 31]; see also [13, 32, 44]). In this task, rats receive a distinct environment reliably paired with nicotine administration. The context alone (no nicotine during testing) comes to evoke an increase in activity relative to controls that only receive exposure to the environment (CS-alone control), to controls that receive equal exposure to nicotine in an unpaired fashion (explicitly unpaired control), and to controls in which the chance of nicotine was similar during CS and non-

CS time periods [5]. The context, a complex polymodal stimulus, is considered the CS. Arguably, the US is the stimulus conditions produced by nicotine [14]. The enhanced activity evoked by the context (termed conditioned response or CR) is thought to reflect a learned association between the context CS and the psychomotor stimulant effects of the nicotine US [5, 32, 39].

Given the importance attributed to associative processes involving tobacco (nicotine) addiction, surprisingly little is known about the environmental factors that modulate acquisition and/or expression of nicotine-conditioned associations. One factor that alters Pavlovian conditioning is the temporal relationship between the onset of the CS and the onset of the US. Manipulating this temporal variable, sometimes referred to as the interstimulus interval (ISI), affects conditioned responding in a wide range of conditioning situations: salivating in dogs [29], auto-shaped key pecking in pigeons [17], nictitating membrane conditioning with rabbits [41], eye-blink conditioning with humans [26], context fear conditioning with rats [3], and ethanol place conditioning in mice [12]. The effect of the ISI on acquisition of nicotine-conditioned hyperactivity or the distribution of this conditioned responding is unknown. Accordingly, the goal of the present set of experiments was to systematically investigate the importance of the ISI in the development of nicotine locomotor conditioning.

2. Materials and methods

2.1. Animals

The subjects were naive male Sprague-Dawley rats (200–225 g on arrival) from Harlan (Indianapolis, IN). They were housed separately in 24 cm × 21.5 cm × 20 cm clear plastic tubs lined with wood shavings. The colony was on a 12 h light:dark cycle; experiments were conducted during the light portion of the cycle. Rats had free access to food and water in the home cages and were handled at least 1 min per day for 3 days before the start of the experiment. The experimental protocols used in this report were approved by the University of Nebraska Institutional Animal Care and Use Committee and were conducted in accordance with the “Principles of Laboratory Animal Care” (NIH publication No. 85–23, revised 1985).

2.2. Drug

Nicotine hydrogen tartrate (Sigma, St. Louis, MO) was mixed in saline (0.9% NaCl) and brought to a pH of 7.0 ± 0.2 with a dilute sodium hydroxide solution. Injections were subcutaneous (SC) at a volume of 1 ml/kg; the dose of nicotine was 0.42 mg/kg base form (ca. 1.2 mg/kg salt form). This nicotine dose reliably produces locomotor conditioning in our laboratory [5].+

2.3. Apparatus

The context CS was one of eight circular chambers made from white PVC pipe. The inside diameter of each chamber was 30.5 cm; the top edge of the chamber was 45 cm from the wire-mesh floor. Each chamber was equipped with two infrared emit-

ter/detector units mounted 4 cm above the mesh floor such that they divided the chamber into four equal sections. Each infrared beam break was automatically recorded by a computer. Activity was defined as the number of infrared beam breaks in each 5 min interval. General illumination of the room was provided by fluorescent ceiling lights; a continuous 80-dB white noise masked external sounds.

2.4. Experiments (background)

Experiment 1 examined the effects of different ISIs. One set of rats received the standard protocol used in past research (e.g. [5]); nicotine administered immediately before each 30 min exposure to the context CS. Other sets received nicotine either 15 min before placement, 15 min after placement, or immediately after being removed from the context. Conditioning was evidenced only in paired rats that had the context CS fully overlap with the effects of the nicotine US (ISI –15 and 0 min). From this experiment it is unclear whether 30 min of overlap with the psychomotor effects of nicotine were required for conditioning, or whether context exposure time in the absence of nicotine prevented expression of conditioning (e.g., extinction [29, 45]). Experiment 2 examined these possibilities by using a paired group that received nicotine 15 min after placement in the context, but context exposure time was increased to 45 min. That is, partial exposure to the context in the absence of nicotine (i.e., opportunity for extinction), yet 30 min of overlap with the psychomotor effects of nicotine. Interestingly, in the drug-free conditioning test the onset of the CR in this group was delayed to the time when the US would have occurred suggesting a timing component to the conditioned association. Because we have never examined a 45 min condition in our laboratory, an alternative possibility is that a 45 min context CS simply controls this pattern of conditioned hyperactivity. Experiment 3 tested this possibility by having a paired and unpaired conditions in which the assigned solution was injected immediately before placement in the context for 45 min.

2.4.1. Experiment 1

Rats were randomly assigned to one of eight groups ($n = 9$ per group): $P_{30}(0)$, $P_{30}(-15)$, $P_{30}(15)$, $P_{30}(30)$, $U_{30}(0)$, $U_{30}(-15)$, $U_{30}(15)$, or $U_{30}(30)$. P or U in the name denotes whether nicotine was paired or unpaired with the context CS, respectively. The subscript number indicates the duration of the conditioning trial (i.e., time in context) in minutes. The number in parentheses indicates the time in minutes between placement in the context and injection (saline or nicotine) on each conditioning trial (i.e., the ISI). Thus, rats in Group $P_{30}(0)$ received an SC injection of nicotine immediately before placement in the locomotor chamber for 30 min. Group $P_{30}(-15)$ received nicotine 15 min prior to placement, Group $P_{30}(15)$ received nicotine 15 min after placement, and Group $P_{30}(30)$ received nicotine immediately upon removal from the context. There were eight placements (i.e., conditioning trials), each separated by 24 h. The other four groups (unpaired) received the same procedure as the comparable paired group except, saline replaced nicotine as the injected solution. To control for exposure to nicotine, rats in the unpaired groups received an injection of nicotine in the home cage approximately 4 h after removal from the locomotor chamber; rats in the paired groups received a saline injection. A drug-free test for conditioning was conducted 24 h after the last conditioning trial.

Each rat was placed in the locomotor chamber for 30 min, and no injection was given on this day. The injection was withheld because the protocol of handling, restraining, injecting, etc. would produce unconditioned change in activity at different time intervals depending on the group. This difference is unacceptable given that we are interested in the temporal pattern of conditioned activity controlled by the context CS.

2.4.2. Experiment 2

Rats were assigned to one of two groups: $P_{45}(15)$ or $U_{45}(15)$ ($n = 14-15$ per group). The conditioning protocol was similar to the comparable group of Experiment 1 except the total time in the context was increased to 45 min. Further, the injection/drug-free test for conditioning conducted 24 h after the last conditioning trial was increased to 45 min.

2.4.3. Experiment 3

Rats were assigned to Group $P_{45}(0)$ or Group $U_{45}(0)$ ($n = 8$ per group). The conditioning protocol was similar to Experiment 2 except injection of the assigned solution occurred immediately before placement in the context for 45 min.

2.5. Data analyses

In Experiments 1 and 3, we compared paired and unpaired activity counts at each ISI value in 5 min intervals for conditioning day 1 (acute effects of nicotine), conditioning day 8 (repeated effects of nicotine), and the injection/drug-free test (conditioned effects of nicotine). Thus, a two-way mixed factorial analysis of variance (ANOVA) was used in which group (paired or unpaired) was the between-subjects factor and interval (5 min intervals) was the within-subject factor. A significant group \times interval interaction prompted post-hoc pair-wise t -tests to determine the source of the interaction. Experiment 2 was conducted in two replications. That is, once the interesting temporal pattern of conditioning was observed in the first replication [$n = 8$ for $P_{45}(15)$; $n = 8$ for $U_{45}(15)$], we conducted another replication to see if the data pattern was reproducible [$n = 6$ for $P_{45}(15)$; $n = 7$ for $U_{45}(15)$]. Accordingly, we treated replication as a factor in the analyses. Thus, the omnibus ANOVA for conditioning and testing was a mixed 3-way factorial with group (paired or unpaired) and replication (first or second) as the between-subject factors and 5 min interval (1-9) as the within-subject variable. Statistical significance was declared at a two-tailed alpha of 0.05 for all tests. If a factor is not mentioned in Section 3, then it was not significant.

3. Results

3.1. Experiment 1

Acute nicotine administration had a transient suppressant effect on activity when injected 15 min before rats were

placed in the context (see Panel A of Fig. 1).¹ For this condition there was a main effect of interval, $F(5, 80) = 29.12$, $P < 0.001$, and a group \times interval interaction, $F(5, 80) = 5.01$, $P < 0.001$. Rats in Group $P_{30}(-15)$ were significantly less active than the $U_{30}(-15)$ rats in the first 10 min of the session, $t's(16) \geq 2.16$, $P's \leq 0.047$. In contrast, repeated nicotine produced locomotor stimulation in the -15 min ISI condition. There was a main effect of interval, $F(5, 80) = 53.57$, $P < 0.001$, and group $F(1, 16) = 19.11$, $P < 0.001$, and a significant group \times interval interaction, $F(5, 80) = 5.07$, $P < 0.001$. Rats in the $P_{30}(-15)$ were more active than the $U_{30}(-15)$ rats in the last 20 min of the session, $t's(16) \geq 3.60$, $P's \leq 0.0024$.

The paired rats in the 0 min ISI condition displayed a similar locomotor pattern (Panel B). For day 1, there was a main effect of interval, $F(5, 80) = 7.62$, $P < 0.001$, and Group $F(1, 16) = 15.53$, $P = 0.001$, and a significant group \times interval interaction, $F(5, 80) = 19.29$, $P < 0.001$. Rats in Group $P_{30}(0)$ were less active than the $U_{30}(0)$ rats in the first 20 min of the session, $t's(16) \geq 12.29$, $P's \leq 0.036$. On day 8, nicotine administration enhanced activity throughout the session as indicated by a significant main effect of group, $F(1, 16) = 27.79$, $P < 0.001$; the main effect of interval was also significant, $F(5, 80) = 50.66$, $P < 0.001$.

Acute administration of nicotine 15 min after placement in the chambers did not affect locomotor activity (see Panel C). Only the main effect of interval was significant, $F(5, 80) = 45.87$, $P < 0.001$, denoting a decrease in activity across time. After repeated administration, rats in Group $P_{30}(+15)$ showed a large increase in activity in the 5 min interval that immediately followed nicotine administration; this enhancement quickly dissipated. There was a main effect of group, $F(1, 16) = 14.12$, $P = 0.002$, a main effect of interval, $F(5, 80) = 31.89$, $P < 0.001$, and a group \times interval interaction, $F(5, 80) = 5.78$, $P < 0.001$. Rats in the paired group were significantly more active than the rats in the unpaired group in intervals 4 and 5, $t's(16) \geq 3.05$, $P's < 0.01$. For rats in the ISI 30 min condition (Panel D), there was only a significant effect of interval on day 1 and day 8 of conditioning, $F's(5, 80) \geq 57.82$, $P's < 0.001$.

Context-evoked hyperactivity was evidenced in paired rats that had a -15 and 0 min ISI [i.e., $P_{30}(-15)$ and $P_{30}(0)$]. Fig. 2 shows the results from the drug-free test for conditioning. For the -15 min ISI condition (see Panel A), there was a main effect of group, $F(1, 16) = 7.34$, $P = 0.015$, and interval, $F(5, 80) = 86.08$, $P < 0.001$, and a significant group \times interval interaction, $F(5, 80) = 2.66$, $P = 0.028$. Post-hoc comparisons revealed that the $P_{30}(-15)$ was more active than the unpaired group only in the first 5 min of the test, $t(16) = 3.01$, $P = 0.008$. For the 0 min ISI condition (see Panel B), there was only a main effect of group, $F(1, 16) = 9.82$, $P = 0.006$, and interval, $F(5, 80) = 80.72$, $P < 0.001$ indicating that paired rats were hyperactive throughout the 30 min session. For the remaining conditions (ISI +15 and +30 min; see Panels C and D), only the main effect of interval was significant, $F's(5, 80) > -40.87$, $P's < 0.001$, denoting that neither procedure produced evidence of conditioning.

¹ Equipment problems resulted in a loss of beam break counts on Trial 1 for one rat in Groups $U_{30}(-15)$, $U_{30}(0)$, $U_{30}(30)$, $P_{30}(0)$, and $P_{30}(15)$. To avoid loss of rats in the overall analyses, we used an estimation procedure to replace missing beam break counts. The estimated value was the average number of beam breaks of the comparable rats at each 5 min interval.

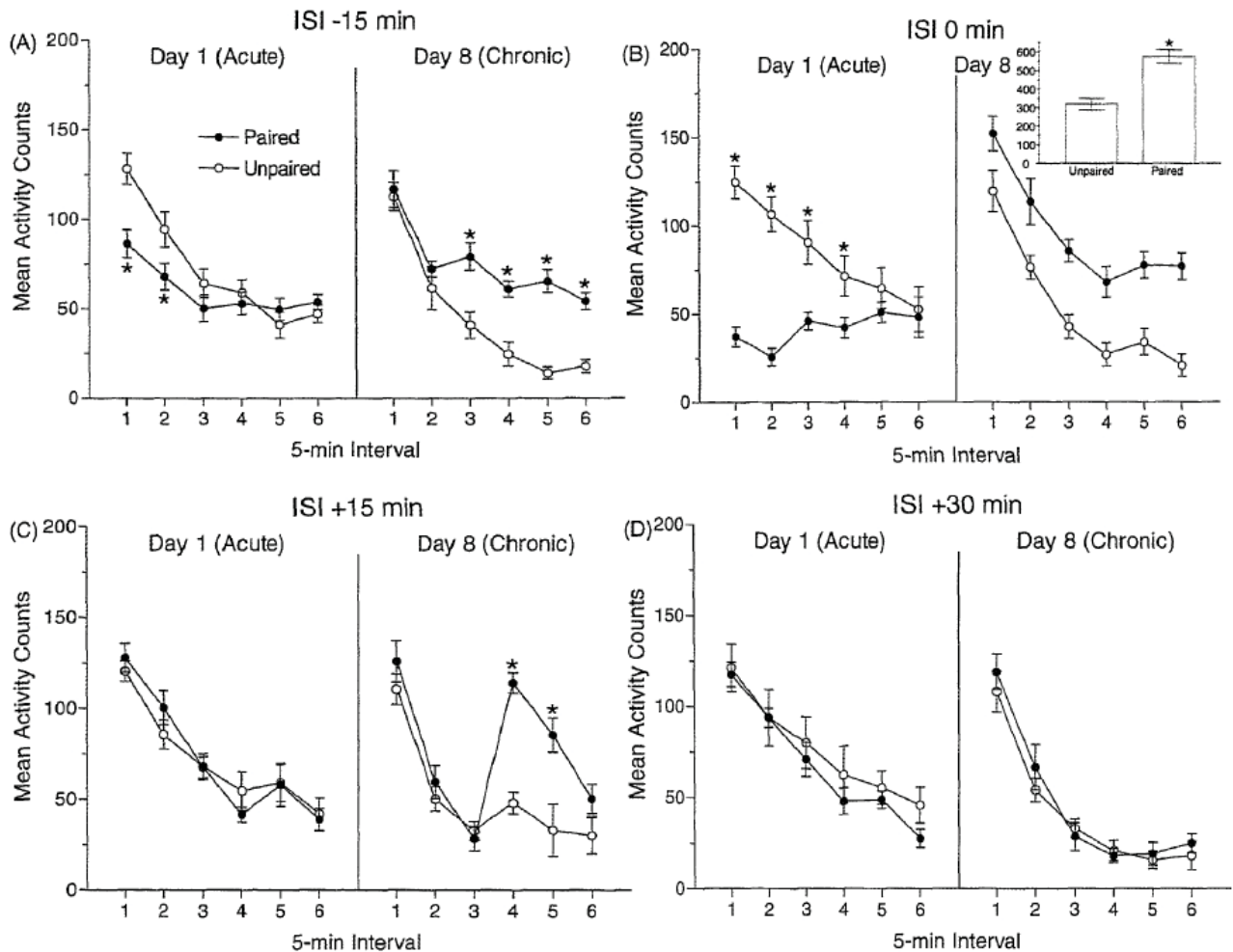


Fig. 1. The mean number of infrared beam breaks (± 1 S.E.M.) on the first and last 30 min conditioning trial for each interstimulus interval (ISI) condition of Experiment 1. The bar graph embedded in Panel B shows margin means for the main effect of group. A group \times interval interaction in the overall analysis prompted pair-wise t -tests comparisons at each interval. * denotes the significant difference ($P \leq 0.05$) in activity between paired and unpaired groups detected by these comparisons.

3.2. Experiment 2

When nicotine was injected 15 min after placement in the chamber, the acute suppressant effect of nicotine was replaced by a distinct pattern of locomotor hyperactivity with repeated exposure. That pattern was characterized by a sharp increase in activity immediately after nicotine was administered (see Fig. 3). As described below, the group factor did not interact with replication on any measure, thus we pooled data from replications for graphic display. The left-most panel of Fig. 3 shows the activity counts for Groups $P_{45}(15)$ and $U_{45}(15)$ on day 1 of conditioning. There was a significant main effect of interval, $F(8, 200) = 66.89$, $P < 0.001$, of group, $F(1, 25) = 9.45$, $P = 0.005$, and a significant group \times interval interaction, $F(8, 200) = 2.37$, $P = 0.018$. No other comparisons were significant. Pair-wise contrasts revealed that Group $P_{45}(15)$ was less active than Group $U_{45}(15)$ on intervals 4 through 7, t 's(27) ≥ 2.77 , P 's ≤ 0.01 , indicating that nicotine injected between intervals 3 and 4 had a suppressant effect on activity. The center panel of Fig. 3 displays activity for the last day of conditioning (day 8). There was a significant main effect of interval, $F(8, 200)$

$= 56.20$, $P < 0.001$, of group, $F(1, 25) = 39.03$, $P < 0.001$, and group \times interval interaction, $F(8, 200) = 7.27$, $P < 0.001$. The only factor including replication that was significant was the replication \times interval interaction, $F(8, 200) = 3.33$, $P = 0.001$. This interaction was driven by higher activity levels, regardless of group, in the first 10 min for replication 2. Pair-wise contrasts prompted by the group \times interval interaction revealed that Group $P_{45}(15)$ was more active than Group $U_{45}(15)$ on intervals 4 through 9, t 's(27) ≥ 3.17 , P 's ≤ 0.004 .

Albeit weaker, the pattern of conditioned responding in this group was remarkably similar to the activity pattern after eight administrations of nicotine. The right-most panel of Fig. 3 shows the activity during the injection/drug-free test for conditioning. Rats in the paired group displayed conditioned hyperactivity that was localized to the latter portion of the test. There was a significant main effect of interval, $F(8, 200) = 67.74$, $P < 0.001$, of group, $F(1, 25) = 12.17$, $P = 0.002$, and a significant group \times interval interaction, $F(8, 200) = 2.48$, $P = 0.014$. No other comparisons were significant. Pair-wise contrasts revealed that Group $P_{45}(15)$ was hyperactive relative to Group $U_{45}(15)$ on intervals 4, 7, 8, and 9, t 's(27) ≥ 2.77 , P 's ≤ 0.01 .

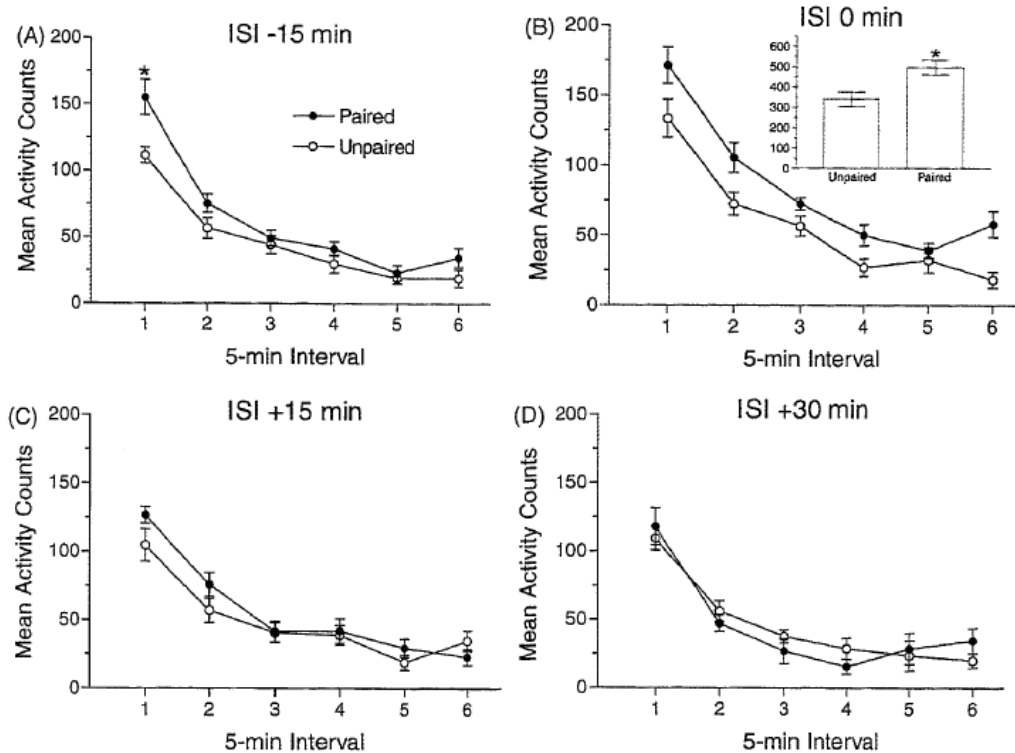


Fig. 2. The mean number of infrared beam breaks (± 1 S.E.M.) in the 30 min injection/drug-free test of conditioning for each interstimulus interval (ISI) condition of Experiment 1. The bar graph embedded in Panel B shows margin means for the main effect of group. A group \times interval interaction in the overall analysis prompted pair-wise t -tests comparisons at each interval. * denotes the significant difference ($P \leq 0.05$) in activity between paired and unpaired groups detected by these comparisons.

3.3. Experiment 3

By the eighth administration of nicotine, rats in Group $P_{45}(0)$ were hyperactive throughout the 45 min session. The left-most panel of Fig. 4 shows the activity on day 1. There was a main effect of interval, $F(8, 112) = 11.75$, $P < 0.001$,

and a significant group \times interval interaction, $F(8, 112) = 6.08$, $P < 0.001$. Contrasts revealed that the $P_{45}(0)$ rats were less active than the $U_{45}(0)$ rats in the first 5 min, $t(14) = 3.60$, $P = 0.003$. This suppression was replaced by weak hyperactivity at interval 8, $t(14) = 2.28$, $P = 0.039$. On day 8 (center panel), there was a main effect of interval, $F(8, 112) = 39.66$,

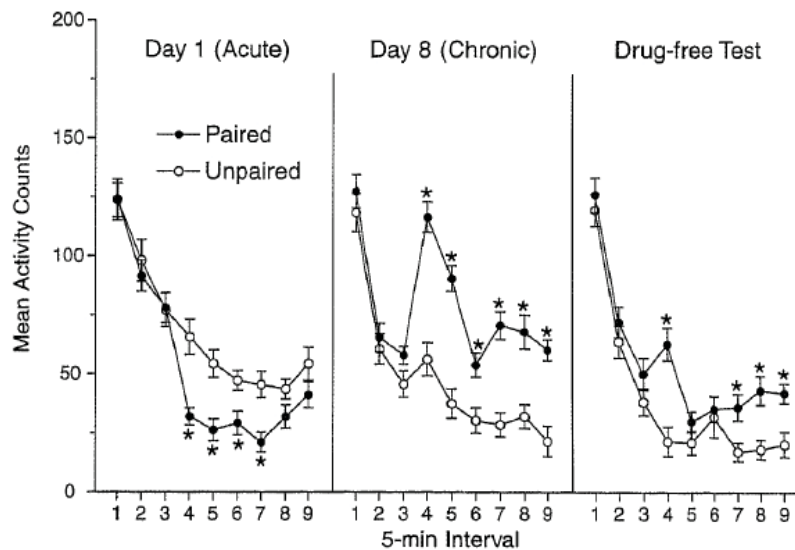


Fig. 3. The mean number of infrared beam breaks (± 1 S.E.M.) on the first and last conditioning trial, and the injection/drug-free test of conditioning for Experiment 2. Each session was 45 min and the ISI on conditioning trials was 15 min. A group \times interval interaction in the overall analysis prompted pair-wise t -tests comparisons at each interval. * denotes the significant difference ($P \leq 0.05$) in activity between paired and unpaired groups detected by these comparisons.

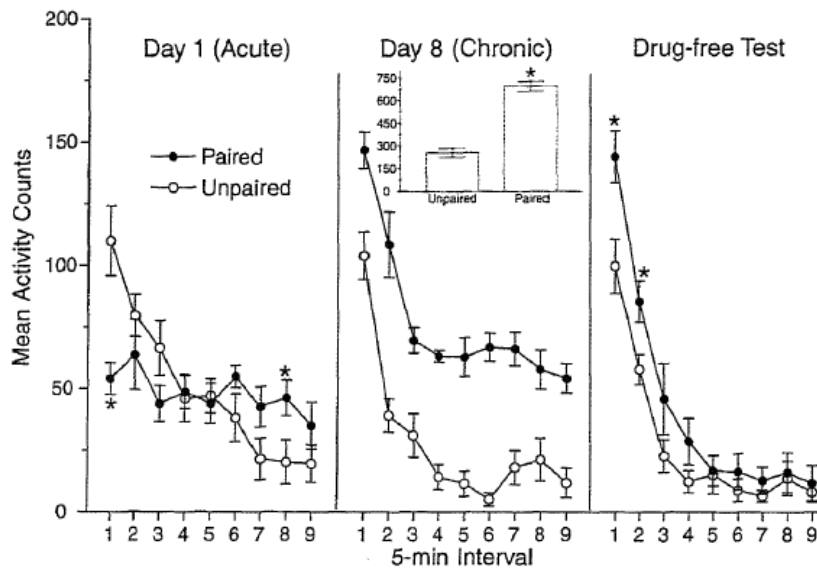


Fig. 4. The mean number of infrared beam breaks (± 1 S.E.M.) on the first and last conditioning trial, and the injection/drug-free test of conditioning for Experiment 3. The bar graph embedded in the center panel shows margin means for the main effect of group. Each session was 45 min and the ISI on conditioning trials was 0 min. A group \times interval interaction in the overall analysis prompted pair-wise *t*-tests comparisons at each interval. * denotes the significant difference ($P \leq 0.05$) in activity between paired and unpaired groups detected by these comparisons.

$P < 0.001$, and group, $F(1, 14) = 99.99$, $P < 0.001$, indicating that paired rats were hyperactive throughout the 45 min conditioning session. Activity counts from the test for conditioning are shown in the right-most panel of Fig. 4. There was a significant main effect of interval, $F(8, 112) = 71.30$, $P < 0.001$, and a significant group \times interval interaction, $F(8, 112) = 2.58$, $P = 0.013$. Conditioned hyperactivity was localized to the first 10 min of the test session, $t's(14) \geq 2.68$, $P's \leq 0.018$.

4. Discussion

Depending on conditions, acute administration of nicotine suppresses locomotor activity in rats; this suppressant effect tends to dissipate with time since administration [5, 11, 13, 42]. Nicotine-induced locomotor suppression is often replaced by activation after repeated exposures to nicotine [5, 11, 13, 22]. This data pattern was replicated in the present report [e.g., Groups $P_{30}(0)$ and $P_{45}(0)$]. These groups showed locomotor suppression early in the first conditioning trial; by the last trial (8th exposure) nicotine produced hyperactivity across the entire session. The injection protocol affected the within-trial pattern of activity for repeated nicotine exposure. For example, in Group $P_{30}(-15)$ hyperactivity was not expressed until later intervals. The lack of hyperactivity early in the session is surprising given that the locomotor effects of repeated nicotine administration are present for at least 45 min (cf. Group $P_{45}(0)$; see also [1, 11, 19]). One possible explanation is that handling, transport to experimental room, and placement in the context disrupts the locomotor effects of nicotine in Group $P_{30}(-15)$. This account is somewhat strained by the very different pat-

tern of activity shown by Groups $P_{45}(15)$ and $P_{45}(15)$ to repeated nicotine exposure because these rats were removed from the chamber, injected, and then returned to the chamber.

Perhaps the most interesting locomotor pattern in the conditioning phase occurred in Group $P_{45}(15)$ [see also $P_{30}(15)$]. In later trials, nicotine produced a sharp increase in activity in the 5 min following administration. This enhanced activity weakened for several intervals before increasing again (see Fig. 3). The initial hyperactivity cannot be explained by the unconditioned activating effects of handling and injecting; comparable unpaired controls did not show a similar increase in activity. At least two factors are likely responsible for this immediate enhancement of activity: (1) unconditioned locomotor stimulant effects of nicotine and (2) conditioned activity controlled by physical (injection, handling, context, interoceptive nicotine cues, etc.) and temporal stimuli (see later) present upon nicotine administration.

The main goal of the present research was to assess whether nicotine-conditioned hyperactivity was sensitive to the temporal arrangement of the context CS and nicotine US. In Experiment 1, if nicotine was administered either 15 min before placement, Group $P_{30}(-15)$, or just before placement in the context, Group $P_{30}(0)$, then an increase in activity relative to controls occurred during testing. The conditioned hyperactivity throughout the 30 min test for Group $P_{30}(0)$ previously published work from our laboratory [5, 7, 30, 31]. Extending the generality of this observation was the evidence for conditioning, albeit weaker, in Group $P_{30}(-15)$. This difference in conditioning is consistent with research in other non-drug Pavlovian conditioning tasks showing weaker conditioned responding when the US onset occurs before the CS onset [25, 29].

The results of Experiment 1 suggest that at least two important factors promote context conditioning. First, 15 min or

less of overlap between the context and the effects of nicotine might not be sufficient for acquisition/expression of a robust CR. Perhaps there needs to be closer to 30 min of overlap. Or, perhaps the effects of nicotine must completely coincide with time in the context [cf. Groups $P_{30}(-15)$ and $P_{30}(0)$]. Second, any delay between context onset and administration of nicotine [cf. $P_{30}(15)$] might weaken/eliminate conditioning via extinction. Extinction (i.e., presentation of the CS without the US) readily weakens expression of conditioned responding [4, 29, 45]. Experiment 2 was designed to test the importance of these two variables. Recall that Group $P_{45}(15)$ of that experiment had the 15 min delay between context onset and nicotine administration as Group $P_{30}(15)$ in Experiment 1. However, there was 30 min of overlap between the context and nicotine (i.e., rats remained in chambers 30 min post-injection). Conditioning in Group $P_{45}(15)$ indicated that a 15 min delay was not necessarily detrimental to development of a locomotor CR. Also, expression of conditioned hyperactivity does not require complete overlap of the context CS with the effects of nicotine.

For conditioned responding to emerge, however, there appears to be a minimal duration of context exposure after nicotine administration—somewhere between 15 and 30 min. Further parametric work is necessary to determine the range of effective values. For instance, perhaps nicotine conditioning, within reasonable constraints, is sensitive to a ratio between context CS exposure and nicotine US exposure (cf. [9, 15, 16]). Group $P_{45}(15)$ had two-thirds (67%) of its time in the context overlap with nicotine. Would conditioning occur under conditions that had similar ratios but different total durations? The present research suggests that context duration will be one of the reasonable constraints. For example, Group $P_{30}(0)$ displayed context-evoked activity across 30 min, whereas Group $P_{45}(0)$ was hyperactive only in the first 10 min of the test. The only difference was that this latter group had 15 min more of context exposure. Alternatively, it might be that longer context CS durations promote CR timing. If so, less conditioned responding in Group $P_{45}(0)$ might reflect better temporal stimulus control of conditioned hyperactivity rather than weaker conditioning.

All groups, except Group $P_{45}(15)$, that displayed evidence for conditioning had hyperactivity concentrated in the early portion or distributed throughout the drug-free test session. Similar to other researchers [13, 32, 44], we interpreted this hyperactivity as reflecting an excitatory Pavlovian conditioned association between the context CS and the psychomotor effects of nicotine [5, 6]. However, the within-trial pattern of conditioned activity of Group $P_{45}(15)$ suggests that, under some circumstances, this simple conditioning account should be revised. In that group, increases in activity in the injection/drug-free test were not observed until after 15 min. This within-trial pattern of nicotine-conditioned hyperactivity is strikingly similar to the pattern seen during later conditioning trials—albeit less pronounced. This suggests that under certain drug-conditioning protocols the conditioned association between the context CS and nicotine US includes temporal in-

formation about the two stimulus events. That is, rats learn ‘when’ the US will occur ([37] see also [15, 16] for alternative ‘timing’ theories).

Recall that rats in Group $P_{45}(15)$ had 15 min of context exposure before receiving a nicotine injection during the conditioning phase. On the test day, this group was placed in the chamber (context CS) for 45 min. Conditioned hyperactivity in the latter two-thirds of the trial cannot be explained by non-temporal cues associated with the injection protocol because handling and injection after initial placement were withheld on the test day. Thus, what remains are the stimulus elements that compose the context and the passage of time. If the context was excitatory, independent of time, then conditioning would occur in the early portion of the test. Further, Experiment 3 eliminated any account suggesting that a locomotor CR emerges later when longer context CS durations are used. Rats exposed to the context for the same duration (45 min), but received nicotine immediately before exposure, were hyperactive only in the early portion of the test. This pattern of conditioned hyperactivity is also consistent with a timing hypothesis; conditioned responding was temporally localized to the time of nicotine administration during the conditioning phase. The suggestion that rats learn about the temporal arrangement between the CS and US is consistent with recent empirical and theoretical work [15, 37]. Empirically, within-session shifts in conditioned responding to changes in the CS-US temporal relation have been reported in such diverse Pavlovian conditioning tasks as rabbit nictitating membrane response [38], conditioned activation in goldfish [5], and one-trial context fear conditioning in rats [3]. The results from the present research reflect the first demonstration of timing of the CR in a nicotine locomotor conditioning preparation.

Reviewers of our earlier published research on nicotine locomotor conditioning raised an important point that could only be indirectly addressed until now. They suggested that hyperactivity in the paired conditions on the drug-free test reflected novelty-induced activity. According to this account, the test day is the first time rats receive exposure to the context in a nicotine-free state. This shift in drug state reflects a change in context or prevents recall of the previous familiarization history [28]. In earlier papers, our enthusiasm for this novelty (or state-dependent familiarization) account was diminished by the lack of evidence for state-dependent learning in rodents, the plethora of published data indicating nicotine typically enhances learning/performance (for reviews see [2, 24]), and the research from our laboratory showing that repeated nicotine exposure does not impair environmental familiarization [6]. However, these arguments were circumstantial. The present work provides direct and unequivocal evidence against the novelty account. On each conditioning trial, rats in the $P_{45}(15)$ condition of Experiment 2 always received 15 min of environmental familiarization in a drug-free state, yet this group still displayed an increase in activity relative to unpaired controls on the test day. One might argue that injecting nicotine immediately after familiarization interfered with neural processes responsible for consolidation and/or lat-

er recall of this familiarization experience. If so, activity in the first 15 min of later conditioning trials should be consistently higher for the paired than the unpaired rats because the environment is familiar only in the latter group. This did not occur (see Fig. 3). Further, a novelty account does not predict the temporal specificity of hyperactivity seen in the P₄₅(15) condition on the test day. Thus, the hyperactivity in nicotine-paired rats of the present and past research in our laboratory more likely reflects the expression of a locomotor CR and not novelty-induced activity.

Finally, to the extent that the present locomotor conditioning task with rats is a good preclinical model for associative-learning processes in humans, the present results suggest that what environmental stimuli enter into associations with nicotine will likely vary depending on their temporal relation to the psychoactive effects of nicotine. Moreover, the timing of conditioned responses evoked by nicotine-associated stimuli (e.g., approach, seeking, craving, etc.) may vary depending on conditioning history. If so, this likely has important implications for associative-learning based approaches to smoking cessation (e.g., cue-exposure therapy). For example, extinction of potential CSs, or counter-conditioning of those cues, might need to be modified to account for the temporal pattern of conditioned responding.

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