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Pharmacology, Biochemistry and Behavior 68 (2001) 135–145

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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# Nicotine-conditioned locomotor activity in rats: dopaminergic and GABAergic influences on conditioned expression

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Received 24 April 2000; received in revised form 7 September 2000; accepted 19 September 2000

**Abstract**

Little is known about the processes that mediate acquisition and expression of conditioned associations between contextual cues and psychomotor effects of nicotine. In four separate experiments using rats, an environment repeatedly paired with nicotine acquired the ability to elicit increases in activity even in the absence of drug. This conditioned effect was sensitive to nicotine dose. Rats that had 0.6 or 1.2 mg/kg nicotine, but not 0.3 mg/kg, paired with the environment were more active than an unpaired control group (Experiment 1). In Experiment 2, control groups eliminated accounts based on nonspecific effects of nicotine and inhibitory conditioning decreasing activity in the unpaired controls of Experiment 1. Pretreatment on the test day with 100 mg/kg of gamma vinyl-GABA (GVG), a compound that inhibits the enzyme required to breakdown GABA, partially blocked the expression of locomotor conditioning without impairing activity in controls (Experiment 3). In Experiment 4, pretreatment on the test day with the dopamine $D_1$ receptor antagonist SCH-23390 (0.03 mg/kg) blocked expression of nicotine-conditioned locomotor activity; the $D_2/D_3$ receptor antagonist eticlopride did not. Thus, the dopamine $D_1$ receptor subtype appears to play a role in context-elicited increases in activity conditioned by nicotine; GABA may also modulate the expression of this conditioned effect. 

*Keywords:* Dopamine; Eticlopride; GVG; Locomotor sensitization; Pavlovian conditioning; SCH-23390; Vigabatrin

Modern drug abuse theories often rely heavily on Pavlovian conditioning processes to account for acquisition, maintenance, and relapse of drug use/abuse (DiChiara, 1995; O'Brien et al., 1992; Robinson and Berridge, 1993; Rose, 1996; Schulties and Koob, 1996; Wise and Bozarth, 1987; but see Tiffany, 1997). For example, Robinson and Berridge (1993) suggested that Pavlovian conditioning processes may control drug seeking, excessive drug taking, and relapse following treatment for the abuse. Theoretical and empirical work on tobacco use also implicates Pavlovian conditioning processes in the maintenance and relapse of chronic cigarette smoking (Carmody, 1990; Fisher et al., 1993; Henningfield et al., 1985, 1996; Rose, 1996; Rose and Levin, 1991; Rose et al., 1993). In this work, the unconditioned stimulus (US) is the psychoactive effects of nicotine; its administration in a moderate tobacco user is repeatedly paired with cues that could serve as conditioned stimuli (CS). These CSs may include throat stimulation, taste and odor of cigarettes, discrete objects such as cigarette pack, matches (lighter), and ash tray, as well as situational (contextual) cues like a bar, living room, smoking area, or vehicle (Lazev et al., 1999; Pritchard et al., 1996; Rose and Levin, 1991; Rose et al., 1993). In fact, Rose and Levin (1991) proposed that methods to attenuate conditioned control of nicotine effects (e.g., extinction and counterconditioning) may decrease relapse rates in smokers. Thus, understanding the basic processes governing the acquisition and expression of CS–nicotine associations will clearly lead to more effective prevention and intervention strategies.

The conditioned locomotor sensitization preparation with rats is often used to examine the behavioral and neurobiological processes governing Pavlovian conditioning with psychomotor stimulants. In the locomotor conditioning preparation, the CS tends to be a multisensory environment (i.e., context) and the US is the drug and its associated stimulus conditions. When the US has psychomotor stimu-

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0091-3057/01/$ – see front matter 
PII: S0091-3057(00)00451-2

lant properties like amphetamine, cocaine, or morphine, conditioning is evidenced by enhanced activity evoked by the previously paired context CS (Anagnostaras and Robinson, 1996; Eikelboom and Stewart, 1982; Neiswander and Bardo, 1987; Stewart, 1992).

Early research had mixed success in finding evidence that contextual stimuli repeatedly paired with nicotine would elicit a conditioned increase in activity. For example, Hakan and Ksir (1988) had a 'conditioning group' that received five context-nicotine conditioning trials. Each conditioning trial included exposure to the context CS for 1 h followed by an injection of nicotine (0.2 mg/kg base). After the injection, each rat was placed back into the context for an additional hour. Relative to various control groups, the authors did not find evidence for nicotine-conditioned locomotor activity. In contrast, Walter and Kuschinsky (1989) found evidence for nicotine-conditioned locomotor sensitization using very different procedures. Their 'conditioning group' received six pairings of a distinct context with nicotine (0.6 mg/kg base). Nicotine was injected immediately before placement in the context for 90 min. With these procedures, rats in the conditioning group were more active than nicotine-equated controls on the test day when both groups received an injection of nicotine (i.e., excitatory Pavlovian conditioning). More recently, Reid et al. (1996, 1998) published further demonstrations of nicotine-conditioned locomotor activity using procedures similar to Walter and Kuschinsky (1989).

In the present paper, we report a series of experiments that further explore the behavioral and neural processes mediating acquisition and expression of a conditioned association between context cues and the psychomotor effects of nicotine. In Experiment 1, we examined whether the acquisition of a context-nicotine association was dose dependent. By using different control groups, Experiment 2 assessed whether group differences in Experiment 1 were due to inhibitory conditioning or nonspecific effects of nicotine rather than excitatory Pavlovian conditioning. Experiment 3 examined the effects of increasing GABA levels on the expression of nicotine-conditioned locomotor activity. Experiment 4 examined the role of the dopamine $D_1$ and $D_2$ receptor subtypes.

## 1. Experiment 1

Because of the relatively limited demonstrations of nicotine-conditioned locomotor effects (Reid et al., 1996, 1998; Walter and Kuschinsky, 1989), very little is known about factors that mediate the acquisition of a context-nicotine association. Thus, the purpose of Experiment 1 was to demonstrate nicotine locomotor conditioning in our laboratory. In doing so, we increased the generality of the effect to a third laboratory and different procedural details. Moreover, we also sought to determine whether the conditioned increase in locomotor activity was sensitive to the magnitude of the US (i.e., nicotine dose). No one has systematically examined the importance of nicotine dose in establishing locomotor conditioning with nicotine.

### 1.1. Method

#### 1.1.1. Animals

The subjects were 56 naive male Sprague–Dawley rats (mean 320 g) from Harlan Sprague–Dawley (Indianapolis, IN) or a breeding colony at the University of Nebraska. They were housed separately in plastic tubs lined with aspen 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/day for 3 days before the start of the experiment.

#### 1.1.2. Drugs

(−)-Nicotine-di-D tartrate (Research Biochemicals International, Natick, MA) was mixed in saline (0.9% NaCl) and then brought to a pH of $7.0 \pm 0.2$ with a dilute sodium hydroxide solution. Injections were subcutaneous (sc) at a volume of 1 ml/kg. Nicotine doses were based on the salt form of the drug.

#### 1.1.3. Apparatus

Activity was automatically recorded in one of four circular chambers made from white PVC pipe. The inside diameter of each chamber was 30.5 cm and the top edge of the chamber was 45 cm from the wire mesh floor. Each chamber was equipped with two infrared emitter/detector units. The infrared units were mounted 4 cm above the mesh floor such that they divided the chamber into four equal sections. Each time the rat broke the infrared beam, a count was automatically sent to an interface and then recorded by a personal computer. Activity was defined as the number of infrared beam breaks across the 30-min session. Fluorescent ceiling lights provided general illumination and a continuous white noise served to mask external sounds.

#### 1.1.4. Procedure

Rats were assigned to one of four groups ($n = 14$ per group). Three of the groups received the locomotor chamber (context CS) paired with 0.3, 0.6, or 1.2 mg/kg nicotine. Rats in the fourth group, an unpaired control group, did not experience nicotine in the presence of the chamber (i.e., injected with saline). Thus, once daily for eight consecutive days, each rat was injected with its assigned solution (0.0, 0.3, 0.6, or 1.2 mg/kg nicotine) and then placed in the locomotor chamber for 30 min. To control for exposure to nicotine, rats in the unpaired control group were divided into two subgroups in which half the rats received an injection of the 0.6 mg/kg nicotine dose in the home cage; the remaining rats were treated with the 1.2 mg/kg nicotine dose. This injection occurred about 2 h after removal from the locomotor chamber. Given that the subgroups did not

statistically differ, we pooled them into one unpaired control group for the purpose of analyses and graphing. Each rat in the remaining three groups received a saline injection in the home cage. To assess whether the context acquired the ability to elicit a conditioned increase in activity by virtue of being paired with nicotine the day after the last conditioning trial, each rat was injected with saline and placed in the chamber for 30 min (i.e., drug-free test).

### *1.1.5. Data analyses*

A repeated measures analysis of variance (ANOVA) was used for overall analysis of the activity data during conditioning trials. Thus, nicotine dose was between groups factor (0.0, 0.3, 0.6, or 1.2 mg/kg) and activity for each trial (1–8) was the repeated measure. Post hoc comparisons prompted by the initial analyses used the Tukey–HSD procedure that controls for Type I error rate. A one-way ANOVA was used for overall analyses of activity during the drug-free test of conditioning. Dunnett's multiple comparison tests were used to determine whether nicotine-paired groups differed from the unpaired control group. Statistical significance was set at a two-tailed alpha of .05 for all tests.

### *1.2. Results and discussion*

Fig. 1A shows the mean level of activity (±1 S.E.M.) for each group across the eight trials. A repeated measure ANOVA yielded a significant main effect of group [$F(3,52) = 6.61$, $P = .001$] and of trial [$F(7,364) = 14.93$, $P < .001$]. The Group × Trial interaction was also significant [$F(21, 364) = 10.51$, $P < .001$]. Subsequent Tukey–HSD contrasts revealed that on Trial 1, only the 1.2 mg/kg nicotine dose significantly depressed locomotor activity relative to the unpaired control group. Rats injected with 0.6 or 1.2 mg/kg nicotine never differed statistically from each other, but were more active than the unpaired control group on Trials 4–8. There were no significant differences between the control rats and rats injected with the 0.3 mg/kg nicotine dose. The rats treated with 0.3 mg/kg nicotine, however, were less active than the 0.6 mg/kg group on Trials 4, 5, and 8, while they were less active than the 1.2 mg/kg group on Trials 5–8.

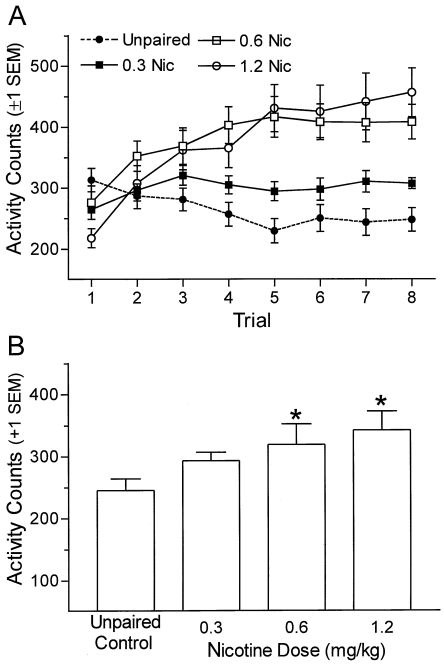


Fig. 1. Panel A shows the mean number of infrared beam breaks (±1 S.E.M.) across the eight conditioning trials for each group in Experiment 1. Panel B displays the mean number of counts for each group during the drug-free test of conditioning. Asterisks denote significant difference ($P < .05$) from the unpaired control using Dunnett's multiple comparison tests.

Fig. 1B shows the results from the drug-free test of conditioning. The one-way ANOVA revealed a significant effect of dose [$F(3,52) = 3.71$, $P = .017$]. Context-elicited activity appeared to increase with the dose of nicotine (i.e., evidence for conditioning). Indeed, subsequent contrasts revealed that the 0.6 and 1.2 mg/kg nicotine-paired groups were more active than the unpaired control group. Thus, like other psychomotor stimulants, a context reliably paired with nicotine appears to elicit an increase in activity even in the absence of drug administration. Importantly, this effect varies with the magnitude of the US (i.e., nicotine dose) similar to more traditional Pavlovian conditioning preparations (Annau and Kamin, 1961; Batsell and George, 1996; Bevins et al., 1997).

## 2. Experiment 2

For over 30 years, the issue of appropriate control procedures for determining whether an effect is the result of Pavlovian conditioning has been heavily debated (Papini and Bitterman, 1990; Rescorla, 1967). The most commonly used control procedure is the explicitly unpaired control. Demonstrations of nicotine locomotor conditioning are no exception to this rule (Reid et al., 1996, 1998; Walter and Kuschinsky, 1989; Experiment 1 of the present report). That control receives exposure to both the CS and US that is identical to the experimental (paired) condition except that the CS and US are separated in time. One criticism of this control procedure is that under certain situations, it may produce inhibitory conditioning (Rescorla, 1967; Stewart and Vezina, 1988). That is, the nicotine US occurs reliably in the absence of the CS (context). Some reports have found that inhibitory conditioning can produce responses that are in direct opposition to the excitatory conditioned responses. In a classic report of this effect, Wasserman et al. (1974) found that pigeons that had the onset of a key-light paired with grain delivery spent more time in the front food/key area than in the rear of the chamber (i.e., excitatory conditioning). In contrast, if grain delivery and key-light presentation were explicitly unpaired, pigeons spent more time in the rear of

the chamber (i.e., evidence for inhibitory conditioning). Wasserman et al. (1974, p. 624) concluded, "subjects may acquire consistent and relatively permanent locomotor and manipulative responses to initially neutral stimuli that predict the presentation or nonpresentation of US."

If inhibitory rather than excitatory conditioning occurred in our situation, then it may be that activity differences in Experiment 1 were due to the unpaired condition exhibiting less activity than the paired condition. A similar account could explain the evidence for conditioning from Reid's laboratory (Reid et al., 1996, 1998). Experiment 2 assessed this inhibitory conditioning account by using two additional control groups: truly-random control (TRC) and CS-alone control. In the TRC group, the probability of the US occurring in the presence of the CS is equal to the probability of the US occurring in the absence of the CS. This control is designed to leave the CS neutral because the CS predicts neither the presence nor the absence of the US (Rescorla, 1967). In the CS-alone control, rats receive comparable exposure to the context CS but never receive the nicotine US. Because no drug is administered to this control, inhibitory conditioning cannot occur. The CS-alone control also provides a baseline in which to assess the nonspecific effects of nicotine on activity. Wasserman et al. (1974) found that CS-alone and TRC groups did not show the locomotor avoidance response evidenced in the explicitly unpaired control pigeons.

### 2.1. Method

#### 2.1.1. Subjects and apparatus

The subjects were 40 naive male Sprague–Dawley rats from Harlan Industries (200–225 g on arrival). All rats were housed separately in stainless steel wire mesh cages. The apparatus was similar to Experiment 1 except eight rather than four circular chambers were used.

#### 2.1.2. Procedure

Rats were assigned to one of four groups ($n = 10$ per group): paired, unpaired, CS-alone, or TRC. The nicotine dose was 1.2 mg/kg. The conditioning phase proceeded as previously described in Experiment 1 for the paired and unpaired groups except that the second injection occurred 6 h after the daily conditioning trial. The CS-alone control received equal exposure to the context CS and the same number of injections, but never experienced the 1.2 mg/kg nicotine US (i.e., all injections saline).

As noted earlier, the TRC group was developed to control for the probabilistic relation between the CS and the US (Rescorla, 1967). To avoid excitatory or inhibitory conditioning, the probability of the US occurring with the CS, $P$(US/CS), should be equal to the probability of the US occurring in the absence of the CS, $P$(US/no CS). The 'no CS' variable is typically defined as a comparable time period as the CS. Neither the CS nor its absence predicts the occurrence of the US in a TRC, thus leaving the CS neutral (but see Benedict and Ayres, 1972; Kremer, 1974; Papini and Bitterman, 1990)). There is at least one difficulty with implementing a TRC in drug conditioning experiments. The TRC group was developed using discrete USs like foot shocks that have more obvious onsets and offsets than drug USs. Nicotine's behavioral and neural actions will extend well beyond the 30-min interval that would define the 'CS' and 'no CS' periods of the present work. For instance, if nicotine was administered in the 30-min time period before the rat was exposed to the context CS, a significant amount of nicotine would still be in the brain during context exposure (Crooks et al., 1997). Does this situation constitute a context CS–nicotine pairing or a no CS–nicotine occurrence? To avoid this situation, 'no CS' and 'CS' periods were lengthened to 6 h. This value was based on recently published work showing low levels of nicotine in the brain 6 h after administration (Crooks et al., 1997). Table 1 shows the procedure received by Rat 1016 in the TRC group. Note that the nicotine US co-occurs with the context CS two out of eight times ($P = .25$); 6 out of 18 times with the 'no CS' periods ($P = .25$). A similar sequence was randomly generated for each rat in the TRC group. Nicotine was injected at the start of the 6-h interval and testing occurred as previously described. Importantly, these procedures also equate US exposure, CS exposure, and number of injections (eight saline and eight nicotine) with paired and unpaired groups.

Table 1
Assignment of nicotine and saline injections for Rat 1016 assigned to the truly random control group of Experiment 2

| | 6-h Intervals | | | |
|---|---|---|---|---|
| | 3 AM–9 AM | 9 AM–3 PM | 3 PM–9 PM | 9 PM–3 AM |
| Trial 1 | – | Context-sal | – | Nic |
| Trial 2 | – | Context-sal | – | Nic |
| Trial 3 | Nic | Context-sal | – | – |
| Trial 4 | – | Context-sal | – | – |
| Trial 5 | – | Context-Nic | – | – |
| Trial 6 | Nic | Context-sal | Nic | – |
| Trial 7 | – | Context-Nic | sal | – |
| Trial 8 | – | Context-sal | Nic | sal |

Abbreviations: sal = saline; Nic = 1.2 mg/kg nicotine injected sc.

### 2.2. Results and discussion

Fig. 2A shows the activity (±1 S.E.M.) for each group across the eight conditioning trials. There was a main effect of group [$F(3,36) = 12.92$, $P < .001$] and a significant Group × Trial interaction [$F(21,252) = 12.02$, $P < .001$]. The main effect of trial was not significant [$F(7,252) = 1.58$, $P = .142$]. Subsequent Tukey–HSD contrasts revealed that on Trial 1, the paired group was less active than controls. This pattern reversed quickly. The paired group was more active than all the control groups for Trials 4–8. The paired group also differed from CS-alone and TRC groups on Trial 3. Recall that on a given conditioning trial, some rats in the TRC group received a context-nicotine

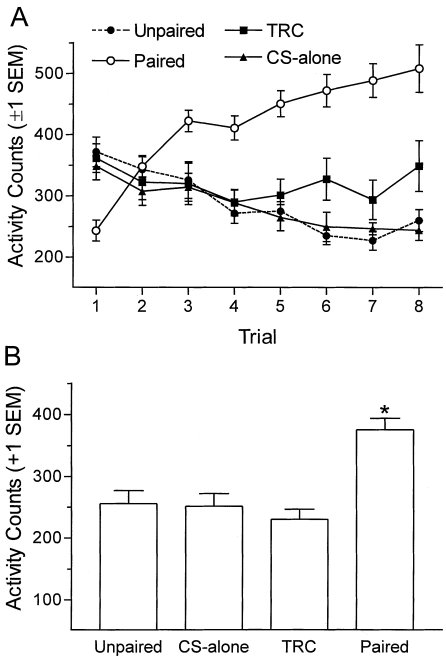


Fig. 2. Panel A shows the mean number of infrared beam breaks (±1 S.E.M.) across the eight conditioning trials for each group in Experiment 2. TRC denotes truly random control group. Panel B displays the mean number of counts for each group during the drug-free test of conditioning. Asterisk denotes significant difference ($P<.05$) from the unpaired control using Dunnett's multiple comparison tests.

pairing. The slightly inflated activity for the TRC group in the later trials is a product of this procedural detail. Regardless, the TRC group never differed statistically from the other two controls.

Fig. 2B shows the results from the drug-free test of conditioning. The data pattern supports an account based on excitatory Pavlovian conditioning in the paired group. The one-way ANOVA found a significant main effect of group [$F(3,39)=11.77$, $P<.0001$]. Dunnett's multiple comparison tests revealed that the paired group was more active than the unpaired group; TRC and CS-alone groups were statistically similar to the unpaired group. This data pattern eliminates an account based on inhibitory conditioning decreasing activity in the unpaired group; that account predicts more activity in TRC and CS-alone groups than in the unpaired group. Enhanced activity in the paired group over the unpaired and TRC groups argues that the reliable co-occurrence of the psychomotor effects of nicotine in the presence of the context CS was important. Finally, exposure to nicotine either in the home cage (unpaired group) or in the home cage and context CS (TRC group) did not inflate activity levels in the locomotor chambers. That is, activity in the CS-alone group during the drug-free test was comparable to the controls that received eight separate injections of nicotine. In sum, the paired group displayed an increase in activity relative to controls; this enhanced activity likely reflects a learned association between the context CS and the locomotor stimulant effects of nicotine.

## 3. Experiment 3

Gamma vinyl-GABA (GVG) selectively inhibits the enzyme required for the breakdown of the transmitter substance GABA (i.e., GABA-transaminase). This inhibition is "irreversible" and results in an increase in brain levels of GABA (Mattson et al., 1995). A recent report found that GVG (Vigabatrin) could prevent nicotine-induced increases of dopamine in the nucleus accumbens (Dewey et al., 1999). Interestingly, in the same report, pretreatment with GVG blocked the acquisition and the expression of a place preference conditioned with nicotine. This latter result (expression) is of particular interest to the present report. In that situation, rats had one distinct environment repeatedly paired with nicotine (0.4 mg/kg base injected sc). A second environment was equally experienced without nicotine. In a subsequent choice test, rats spent more time in the nicotine-paired environment. Pretreatment with GVG (18.75–150 mg/kg) 2.5 h before the choice test blocked this preference for the paired compartment. In Experiment 3, we sought to test whether pretreatment with GVG before the test could block the expression of a nicotine-conditioned increase in activity elicited by the context CS.

### 3.1. Method

#### 3.1.1. Procedure

The subjects were 88 naive male Sprague–Dawley rats (200–225 g on arrival). Animal housing and experimental apparatus were identical to Experiment 2. GVG (gift from Hoechst Marion Roussel, Bridgewater, NJ) was dissolved in saline and injected intraperitoneal (ip) at a volume of 1 ml/kg. GVG doses were based on the salt form of the drug. In the conditioning phase, rats were randomly assigned to the paired or unpaired condition ($n=44$ per condition). The nicotine dose was 1.2 mg/kg. The conditioning phase proceeded as previously described in Experiment 1 for paired and unpaired groups except that the control sc injection of nicotine for the unpaired group occurred approximately 8 h after the daily conditioning session; the paired group received a saline injection at this time. Moreover, each rat received an ip injection of saline 2.5 h before each conditioning trial. This protocol prevented the injection of GVG or vehicle from being novel on the test day. On the test day, rats received an ip injection of one of four concentrations of GVG (0, 50, 100, or 200 mg/kg) 2.5 h before a sc saline injection and subsequent placement in the locomotor chamber for 30 min (i.e., the nicotine-free test for conditioning). Thus, the design on the test day was a $2\times4$ factorial with group (paired or unpaired) as one factor and GVG dose as the second factor ($n=11$ per cell). Selection of injection protocol and doses for GVG were based on published reports showing the effectiveness of this protocol to block the behavioral effects of nicotine and cocaine (Dewey et al., 1998, 1999).

### 3.1.2. Data analyses

As in the previous experiments, activity across the eight conditioning trials was analyzed using a repeated measure ANOVA. For the activity data on the drug-free test day, we first report the outcome of the omnibus two-way ANOVA with condition (paired versus unpaired) as one factor and GVG dose as the second factor. We then conducted planned contrasts that required us to compare the control groups (paired or unpaired without GVG) to their respective GVG-treated groups. To do so, we conducted separate one-way ANOVAs for the paired and the unpaired conditions followed by Dunnett's contrasts.

### 3.2. Results and discussion

Fig. 3A shows the activity (±1 S.E.M.) for the paired and unpaired groups across eight conditioning trials.[1] There was a main effect of group [$F(1,86) = 86.73$, $P < .001$], a main effect of trial [$F(7,602) = 7.13$, $P < .001$], and a significant Group × Trial interaction [$F(7,602) = 158.62$, $P < .001$]. Subsequent contrasts revealed that on Trial 1, the paired rats were less active than controls. This pattern reversed quickly. Paired rats were more active than controls on Trials 3–8. To determine whether activity before the conditioning test was comparable for each subset of rats in the paired condition (i.e., 0, 50, 100, and 200 mg/kg GVG), we conducted a one-way ANOVA on Trial 8 activity; a separate ANOVA was conducted for the unpaired conditions. Activity on Trial 8 was statistically similar for each subset of rats in the paired and unpaired conditions [$F$'s < 2.31, $P$'s > .091]. Thus, differences on the test day cannot be attributed to differences on the last conditioning trial.

Fig. 3B shows the results from the nicotine-free test for conditioning. The two-way ANOVA on the test data revealed a main effect of group [$F(1,80) = 56.52$, $P < .001$] and a main effect of GVG dose [$F(3,80) = 56.81$, $P < .001$]. The Group × GVG Dose interaction approached statistical significance [$F(3,80) = 2.61$, $P = .057$. Subsequent planned contrasts assessed whether the control groups (paired or unpaired) differed from their respective GVG-treated groups. For the unpaired condition, the highest dose of GVG significantly decreased locomotor activity relative to saline-treated controls, indicating motor-impairment at the 200 mg/kg dose of GVG. In the paired condition, the groups treated with the 100 and 200 mg/kg doses of GVG were significantly less active than saline-treated paired rats. Despite this decrease relative to paired controls, the paired rats treated with 100 mg/kg GVG were still significantly more active than vehicle-injected rats in the unpaired condition ($P = .029$). This data pattern indicates that the expression of nicotine-conditioned locomotor activity was attenuated by 100 mg/kg of GVG.

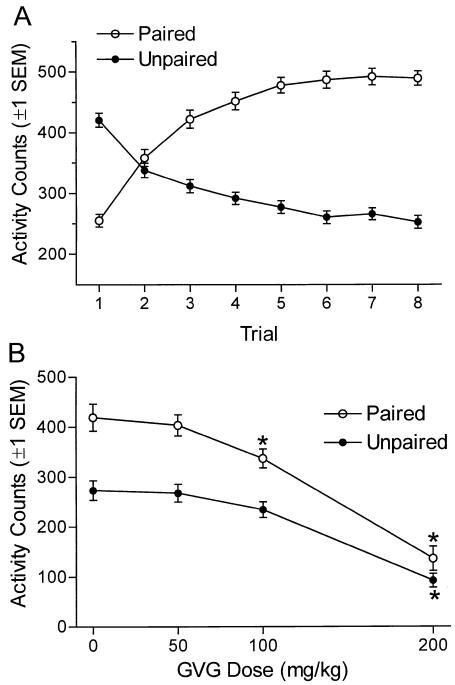


Fig. 3. Panel A shows the mean number of infrared beam breaks (±1 S.E.M.) across the eight conditioning trials for the paired and unpaired conditions in Experiment 3. Panel B displays the mean number of counts for each of the unpaired and paired groups pretreated with saline or one of three doses of γ-vinyl-GABA (GVG) before the test of conditioning. Asterisk denotes significant difference ($P < .05$) from the corresponding saline-treated control using Dunnett's multiple comparison tests.

## 4. Experiment 4

Reid et al. (1996, 1998) suggested that the conditioned component of locomotor activity induced by nicotine was mediated by an increase in dopamine in the nucleus accumbens. Thus, we were surprised by the inability of GVG to block the expression of nicotine-conditioned locomotor activity given previous research demonstrating GVG blockade of the expression of nicotine place conditioning and nicotine-induced increases in dopamine in the nucleus accumbens (Dewey et al., 1999). Experiment 4 more directly examined the role of the dopaminergic system in the expression of nicotine-conditioned locomotor activity using the dopamine $D_1$ receptor antagonist SCH-23390 (Seeman and Niznik, 1988; Seeman and VanTol, 1994) and the dopamine $D_2/D_3$ receptor antagonist eticlopride (Boundy et al., 1993; Köhler et al., 1986).

### 4.1. Method

#### 4.1.1. Procedure

The subjects were 100 naive male Sprague–Dawley rats (200–225 g on arrival). Housing and apparatus were

[1] A power surge occurred on Trial 7 resulting in the loss of activity counts for three rats in the paired group and two rats in unpaired group. We estimated activity for this trial by taking the average number of counts on Trials 6 and 8 separately for each rat. Analyses and figures reflect this estimation procedure.

unchanged. *R*(+)-SCH-23390 hydrochloride and *S*(−)-eticlopride hydrochloride (RBI/Sigma, Natick, MA) were dissolved in saline and injected ip at a volume of 1 ml/kg. All doses were based on the salt form of the drug.

In the conditioning phase, rats were randomly assigned to the paired ($n=50$) or unpaired ($n=50$) groups. The conditioning phase proceeded as previously described except that each rat received an ip injection of saline 30 min before each conditioning trial so that on the test day, the antagonist or vehicle injection would not be novel. On the test day, rats received an ip injection of one of two concentrations of SCH-23390 (0.01 or 0.03 mg/kg), one of two concentrations of eticlopride (0.03 or 0.1 mg/kg), or saline 30 min before the drug-free test. The drug-free test for conditioning was as previously described. Each rat received a sc saline injection immediately before placement in the locomotor chamber for 30 min. Half the rats assigned to each solution had previously received nicotine paired with the environment (paired group); the remaining rats were from the unpaired group.[2] Selection of injection protocol and doses of antagonists were based on published reports from our laboratory and others (Bardo et al., 1993; Besheer et al., 1999).

### 4.2. Results and discussion

For the paired and unpaired groups across the eight conditioning trials (see Fig. 4A), there was a main effect of group [$F(1,97)=82.21$, $P<.001$], a main effect of trial [$F(7,679)=13.05$, $P<.001$], and a significant Group × Trial interaction [$F(7,679)=100.43$, $P<.001$]. Contrasts revealed that the paired group was less active than the unpaired group on Trial 1. From Trial 3 to Trial 8, this pattern was reversed; the paired group was more active than the unpaired group. As in Experiment 3, to determine whether activity before the conditioning test was comparable for each subset of rats, we conducted separate one-way ANOVAs on Trial 8 activity for the paired and unpaired conditions. Activity on Trial 8 was statistically similar for each subset of rats in the paired and unpaired conditions [$F$'s $\leq 1.04$, $P$'s $\geq .396$], indicating that differences on the test day cannot be due to differences on the last conditioning trial.

Fig. 4B shows the results from the nicotine-free test of conditioning for the saline and the SCH-23390 treated rats in the paired and unpaired groups. The two-way ANOVA on the test data revealed a main effect of group [$F(1,53)=18.68$, $P<.001$] and a main effect of SCH-23390 dose [$F(2,53)=5.51$, $P=.007$]. The Group × Drug interaction was not significant ($F<1$). Planned Dunnett's contrasts determined whether the saline control groups (paired or unpaired) differed from their respective SCH-23390 treated groups. For the unpaired controls, SCH-23390 treatment did not alter locomotor activity. In the paired conditions, however, rats treated with the 0.03 mg/kg doses of SCH-23390 were significantly less active than saline-treated paired rats. Moreover, their activity level was statistically similar to vehicle-treated unpaired controls ($P=.990$). This data pattern suggests dopamine $D_1$ receptor antagonism blocks expression of nicotine-conditioned locomotor activity.

Fig. 4C shows the results from the nicotine-free test for conditioning in the saline and the eticlopride treated rats in the paired and unpaired groups. The saline-treated rats were

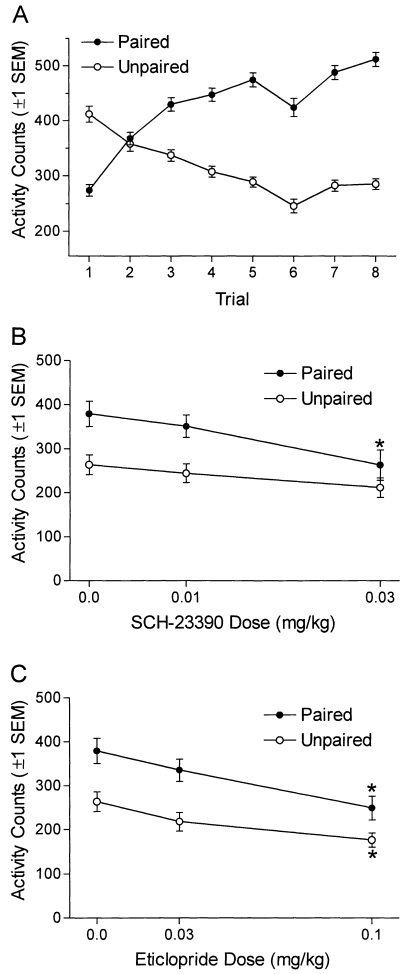


Fig. 4. Panel A shows the mean number of infrared beam breaks (±1 S.E.M.) across the eight conditioning trials for the paired and unpaired conditions in Experiment 4. Panel B displays the mean number of counts for each of the unpaired and paired groups treated with saline or one of two doses of SCH-23390 before the test of conditioning. Panel C displays the mean number of counts for the unpaired and paired groups treated with saline or one of two doses of eticlopride before the test. Asterisk denotes significant difference ($P<.05$) from the corresponding saline-treated control using Dunnett's multiple comparison tests.

[2] The sample size was 10 for each group upon initial random assignment. However, one rat from the paired group that received 0.03 mg/kg SCH-23390 was lost due to experimental error. Data analyses and figures reflect this loss. On conditioning Trial 2, infrared beam failure resulted in a loss of data for one rat from the paired group and one rat from the unpaired group. We estimated activity for this trial by taking the average number of counts on Trials 1 and 3. Analyses and figures reflect this estimation procedure.

the same as those used for analyses and graphics displayed in Fig. 4B. The two-way ANOVA revealed a main effect of group [$F(1,54) = 27.67$, $P < .001$] and a main effect of eticlopride dose [$F(2,54) = 10.59$, $P < .001$]. The Group $\times$ Drug interaction was not statistically significant ($F < 1$). Planned contrasts revealed that the highest dose of eticlopride in the unpaired group significantly decreased locomotor activity relative to saline-treated controls, indicating motor-impairment at the 0.1 mg/kg dose of eticlopride. The paired rats displayed a similar pattern. Rats treated with the 0.1 mg/kg doses of eticlopride were significantly less active than saline-treated paired rats. Thus, the dopamine $D_2/D_3$ receptor antagonist eticlopride decreased nicotine-conditioned increases in activity only at a dose that decreased locomotor activity in controls.

## 5. General discussion

Acute treatment with nicotine suppresses locomotor activity in rats (Clarke and Kumar, 1983; Stolerman et al., 1973, 1995). Depending on the conditions of the experiment, this suppression can be quickly replaced by locomotor activating effects (Clarke and Kumar, 1983; Ksir, 1994; Ksir et al., 1987). This activity pattern was replicated in each experiment of the present report. The 1.2 mg/kg nicotine dose, but not the 0.6 or 0.3 mg/kg dose, initially suppressed locomotor activity relative to controls. The two higher doses, however, produced comparable increases in locomotor activity (i.e., sensitization) by the last conditioning trial. In the drug-free test of conditioning, rats that had the locomotor chambers paired with nicotine (0.6 or 1.2 mg/kg) were more active than controls that received similar exposure to nicotine and the context CS. Notably, the demonstration of conditioning does not require locomotor suppression by nicotine on the first treatment day. The 0.6 mg/kg dose of nicotine produced conditioned locomotor sensitization in the drug-free test, but did not significantly suppress activity in the first 30-min session.

The most widely used control procedure to evaluate the locomotor conditioning effects of psychomotor stimulants, including nicotine, provides comparable drug and context exposure separated in time (i.e., explicitly unpaired control). This situation leaves open the possibility that inhibitory conditioning in this unpaired control (i.e., decrease in activity) may be responsible for the differences between paired and unpaired groups on the test day (Stewart and Vezina, 1988; Wasserman et al., 1974). The results of Experiment 2 eliminate this account. According to an inhibitory conditioning account, the CS-alone control should be more active than the unpaired control group in the test because inhibitory conditioning cannot occur in the CS-alone control. This did not occur; activity was statistically comparable between groups. Moreover, the group that controlled for the predictive relation between the co-occurrence of the nicotine US with the CS and no CS periods (TRC group) did not differ from the CS-alone and unpaired groups. This outcome further argues against an inhibitory conditioning account. Thus, like other drugs of abuse, the behavioral activating effects of nicotine can come under environmental control (Anagnostaras and Robinson, 1996; Neiswander and Bardo, 1987; Stewart, 1992).

There is at least one alternative account to excitatory conditioning that we did not directly assess in the present report. That account suggests that nicotine interferes with environmental familiarization processes. Thus, on the test day when nicotine is no longer administered, the test context for the paired rats is novel relative to controls that do not experience nicotine in the context (cf. Ahmed et al., 1995). Rats tend to be more active in a novel than in a familiar environment (see Trial 1 versus Trial 8 for unpaired rats in Fig. 3A). Albeit plausible, our enthusiasm for this novelty account is diminished for several reasons. First, previously published reports tend to find that chronic nicotine either facilitates or has no effect on learning processes. When nicotine interferes with learning, the task tends to be susceptible to proactive interference (e.g., Dunnett and Martel, 1990). That is, what is first learned impairs later learning. Environmental familiarization is clearly a learning phenomenon. However, there does not appear to be the opportunity for proactive interference. What is learned about the context on the first exposure is the same as what should be learned on subsequent exposures.

A second reason for our decreased enthusiasm for the novelty account comes from a recently published report from our laboratory testing whether chronic nicotine interfered with environmental familiarization (Bevins et al., in press). This test took advantage of the fact that rats interact more with an object when the environment is familiar than when it is novel. Briefly, rats were treated once daily for 14 days with nicotine (0.6 mg/kg, sc). On the next 2 days, rats received exposure to a novel environment (2 min/day) following treatment with nicotine. On the following day (test), rats were placed back in the environment without nicotine, but an object was present in the environment for the first time. These rats interacted more with the object than controls for which the environment was novel. Further, object interaction in this group was statistically comparable to a control that was familiar with the environment (i.e., environment exposure without nicotine). In short, chronic nicotine did not impair environmental familiarization.

The results of the drug-free test corroborate and provide an important extension of recent research also demonstrating nicotine-conditioned locomotor sensitization to a context CS (Reid et al., 1996, 1998; Walter and Kuschinsky, 1989). For example, the present work increases the generality of this finding to a different laboratory and conditioning procedures. In the present report, the time in the paired context was much shorter than previous reports (30 min), the dose of nicotine was lower (1.2 mg/kg salt form or approximately 0.42 mg/kg free base), and the paired environment differed vastly (circular PVC chamber versus rectan-

gular Plexiglas chamber and wire cages). Moreover, in Experiment 1, we provide the first published report that this conditioned increase in activity is sensitive to the dose of nicotine. This sensitivity of conditioning to US magnitude is consistent with a large set of Pavlovian conditioning literature using more traditional learning preparations (e.g., Annau and Kamin, 1961; Batsell and George, 1996; Bevins et al., 1997).

Perhaps the most interesting difference in procedure was in the test for conditioning. Reid et al. (1996, 1998; see also Walter and Kuschinsky, 1989) injected all rats with nicotine during the test. The greater activity in the paired rats presumably reflects a "summation" of the unconditioned activity from nicotine and the conditioned activity elicited from the paired environment. In the present experiment, the test was conducted in the absence of nicotine. Thus, the greater activity in the paired rats reflects conditioned activity elicited by the paired environment. Theories of drug abuse often rely on Pavlovian conditioning processes to explain drug craving, seeking, and relapse following sustained abstinence (O'Brien et al., 1992; Robinson and Berridge, 1993; Schulties and Koob, 1996). One important implication of the nicotine locomotor conditioning research conducted to date is that environmental stimuli reliably paired with nicotine administration (e.g., vehicle, designated smoking area, or living area) may elicit conditioned responses like craving in the absence of drug as well as alter unconditioned responses to the drug after administration.

Reid et al. (1996, 1998) suggested that the expression of nicotine-conditioned locomotor activity to a paired context CS was mediated by dopamine. The results of the present report support this conclusion. Indirect support comes from Experiment 3. Pretreatment with 100 mg/kg GVG before the drug-free test partially blocked expression of nicotine-conditioned activity before impairing locomotor activity in controls (200 mg/kg). In a subsequent pilot study, we found that an intermediate dose of 150 mg/kg GVG also produced motor impairment (decreased number of infrared beam breaks). Dewey et al. (1999) found that 100 mg/kg of GVG completely blocked nicotine-induced dopamine release in the nucleus accumbens and even lower doses blocked expression of nicotine-conditioned place preference. The effect of GVG on nucleus accumbens dopamine is likely mediated by inhibitory GABAergic input to the ventral tegmental area (Gerasimov et al., 1999). Of empirical interest will be experiments that more directly assess the dopaminergic system in the place-conditioning situation employed by Dewey et al. (1999). Will selective dopamine antagonists block expression of nicotine-conditioned place preference? Further, GVG increases brain levels of GABA. It will be of interest to determine whether antagonists specific for different GABA receptor subtypes will block expression of nicotine conditioning in the place preference and locomotor task (cf. Gerasimov et al., 1999; Jackson et al., 2000). Although one should be cautious when comparing experiments between laboratories, we find it very interesting that the expression of nicotine-conditioned locomotor activity was less susceptible to blockade by GVG than expression of a nicotine-conditioned place preference (Dewey et al., 1999). Little is known about the behavioral or neurobiological processes that mediate the conditioned effects of nicotine. The difference in the effectiveness of GVG in the two learning preparations suggests a dissociation in the processes that mediate the nicotine-conditioned expression of locomotor activity versus place preference.

More direct support for the role of dopamine in the expression of nicotine-conditioned locomotor activity comes from the study employing dopamine receptor antagonists (Experiment 4). The dopamine $D_1$ antagonist SCH-23390 blocked expression of nicotine-conditioned locomotor activity without significantly altering activity levels in controls. This result appeared specific to the $D_1$ receptor subtype. Eticlopride, an antagonist slightly more selective for the dopamine $D_2$ receptor over the $D_3$ receptor, did not block expression of nicotine-conditioned increases in activity at a dose that did not impair locomotor behavior in controls. This outcome does not preclude the possibility that dopamine $D_2$-like receptors are involved in the expression of nicotine-conditioned locomotor activity. Further experimentation with other selective ligands (e.g., sulpiride or L-741,626) for the $D_2$ receptor subtype will be necessary.

In sum, we found in four separate experiments that an environment repeatedly paired with nicotine acquired the ability to elicit an increase in activity in the absence of any drug. This conditioned effect was sensitive to the dose of nicotine (magnitude of the US). Control groups eliminated accounts based on nonspecific effects of nicotine and inhibitory conditioning. Pretreatment with GVG before the conditioning test partially blocked the expression of the nicotine-conditioned locomotor stimulant effects elicited by the paired environment. The expression of nicotine-conditioned locomotor sensitization was blocked by pretreatment with SCH-23390, but not by eticlopride. At present, our results, combined with previous research, suggest that postsynaptic dopamine $D_1$ receptors in the nucleus accumbens may play a role in mediating the increase in activity elicited by a context CS that has been reliably paired with a nicotine US; GABAergic input to the ventral tegmental area may modulate this conditioned effect.

## Acknowledgments

Grants from the National Institute on Drug Abuse (DA-11893), the Nebraska Cancer and Smoking Disease Research Program, and the University of Nebraska-Lincoln Research Council supported the research in the present report. The Vigabatrin (GVG) was a gift from Hoechst Marion Roussel. We thank Roger Dooley for designing and constructing the automated infrared emitter/detector system used to collect the activity data. We are also grateful to Todd Hinze for his technical assistance, to Michael Bardo and

Matthew Palmatier for their thoughtful comments on an earlier version of this report, and to Eliot Hearst for donating the white noise generator.

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