Published in final edited form as:

J Gen Psychol. 2013 ; 140(3): 187–203. doi:10.1080/00221309.2013.785929.

Acquisition, Extinction, Recovery, and Reversal of Different Response Sequences Under Conditional Control by Nicotine in Rats

Joseph R. Troisi II, Ph.D.

Saint Anselm College

Abstract

Complex voluntary behaviors occur in sequence. Eight rats were trained in an operant procedure that used nicotine and non-drug (saline) states as interoceptive cues that signaled which of two behavioral sequences led to food reward. The distal and proximal responses in the chain were always maintained on variable interval 30-sec and fixed ratio-1 schedules, respectively, and rate differences between the responses were used as the dependent variable. Extinction and reversal training was conducted. Distal response rates were significantly greater than proximal response rates during training, testing, extinction, and reversal learning. These data suggest that (a) nicotine can establish interoceptive control over different response sequences, and (b) extinction of one response sequence may be state-dependent. The clinical relevance of extinction of complex behavioral repertoires such as drug-seeking and drug-taking behavior that are evoked by specific interoceptive cues is addressed in regard to drug abuse treatment and relapse.

Keywords

conditional discrimination; drug discrimination; drug-seeking behavior; extinction; heterogeneous chain; interoception; nicotine; rats; response sequences; relapse

Human drug-seeking and drug-taking rituals involve the emission of a complex series of topographically different voluntary (i.e., operant) responses that eventuate in drug self-administration and drug reward. For instance, drug abusers seek out a drug, prepare the drug, and then self-administer the drug. Intravenous drug abuse, crack cocaine abuse, tobacco smoking, and alcohol consumption have specific rituals associated with the drug (e.g., Conklin & Tiffany, 2002; Siegel, 2005; Troisi, in press). Animal laboratory models have emerged to simulate human drug self-administration. In a standard rodent model of drug self-administration, lever-pressing is reinforced on a two-link (i.e., second-order) schedule of reinforcement (Everitt & Robbins, 2000; Schindler, Panlilio, & Goldberg, 2002 for reviews) in which the completion of one schedule (e.g., fixed interval) is followed by a second schedule (e.g., fixed ratio). Completion of the second schedule on the same response manipulandum (the lever) is followed by a brief exteroceptive stimulus cue that is paired

Correspondence: Joseph R. Troisi II, Department of Psychology, Saint Anselm College, 100 St. Anselm Dr., Manchester, NH 03102
jtroisi@anselm.edu.

Conflicts of Interest: The author has no conflicts of interest, financial or otherwise.

with the drug reinforcer. More complex behavioral repertoires established with the operant *heterogeneous chain* (see next) have recently emerged to more ecologically simulate human drug-seeking and drug-self-administration in rats (e.g., Pelloux, Everitt, & Dickinson, 2007; Lu, Li, Hou, Chen, Chi, & Liu, 2010).

The heterogeneous operant chain (D'Andrea, 1969; Lattal & Crawford-Godbey, 1985) requires the emission of at least two topographically different responses in a sequence. Such responses are linked by an exteroceptive stimulus (e.g., light or tone). For example, a lever press response (the *distal response*) might be followed by a pole push or a nose poke response (the *proximal response*). When the distal response schedule is completed, an exteroceptive cue (i.e., discriminative stimulus - S^D) is presented that signals reinforcement availability (S^{r+}) (e.g., food pellet, water, drug delivery) if the proximal response schedule is completed. Some rather sophisticated behavioral repertoires can be established in rats with this methodology (see Pierrel & Sherman 1963, "*Barnabus the rat with college training*") that should be more often applied to the study of drug abuse as compared to the standard two-link second order schedules described earlier. For instance, Lu et al. (2010) required rats to respond on a nose-poke manipulandum that opened a door allowing self-administration of 120 $\mu\text{g/kg}$ of heroin contingent on crossing an alley. In this example the nose-poke response might be analogous to "drug seeking behavior" whereas crossing the alley is the self-administration response.

In addition to the rewarding effects that contribute to drug self-administration, drugs also produce a variety of interoceptive "subjective" effects that, when methodologically arranged, can also function as reliable interoceptive cues (i.e., operant discriminative - S^D s) that predict the availability of reinforcer if a specified behavior occurs. This method is commonly known as *drug discrimination* in the behavioral pharmacology literature (e.g., Colpaert & Koek, 1995). There is good functional similarity between exteroceptive (visual or auditory) and interoceptive (drug states) stimulus control phenomena (Troisi, 2003a for brief summary; cf., Bevins and Murray, 2011 for Pavlovian analogs) in the regulation of behavior. It has previously been suggested that a variation of the operant drug discrimination paradigm may have clinical utility in simulating the manner in which interoceptive stimuli (e.g., emotions, stress, hunger, fatigue etc.) gain antecedent stimulus control over drug-taking behavior (Troisi, 2003b; 2006; 2011; Troisi, Bryant, & Kane, 2012; cf., Bevins & Murray, 2011 for Pavlovian interpretations). For example, Panlilio, Thorndike, and Schindler (2008) demonstrated that interoceptive changes in blood-drug levels may function as operant discriminative stimuli that set the occasion for the organism to self-administer the drug. More demonstrably, Beardsley, Anthony & Lopez (1992) showed that phencyclidine (PCP) functioned effectively as an interoceptive cue that signaled the availability of ethanol-reinforcement in rats. In that study, PCP signaled which of two levers led to EtOH reward. Under saline the responses choice was the opposite.

This laboratory (Troisi, 2003a,b; 2006; 2011; Troisi et al, 2012; Troisi, LeMay, & Järbe, 2010) has systematically investigated aspects of extinction of operant behavior evoked by the discriminative stimulus effects of nicotine, including: spontaneous recovery, reinstatement, context renewal, transfer, and reversal learning. Thematically, drug treatment related issues pertaining to extinction of behavior evoked by interoceptive drug-related cues

have been of particular theoretical importance. For instance, drug treatment strategies involving extinction of exteroceptive drug-related stimuli (*cue exposure therapy*) have been equivocal; this is likely attributable to spontaneous recovery (the return of responding following a delay after extinction), reinstatement (return of responding following mere presentation of the reward), and context renewal (return of responding in a different setting) of stimulus control - phenomena commonly reported in the animal learning literature (Conklin & Tiffany, 2002). With this in mind, extinction of operant behavior under specific stimulus control of interoceptive states may elucidate some of the problems posed for cue exposure therapy (see Troisi, in press; cf., Bevins and Murray, 2011 for a Pavlovian perspective).

Theoretically, complex sequences of behaviors may be evoked by changes in interoceptive states that are associated with the primary reinforcer. To the author's knowledge, the analysis of the discriminative stimulus effects of drugs has not made use of a heterogeneous operant chain procedure. As stated by Kelleher and Gollub (1962, p 544), "The stimuli in a chain may of course be exteroceptive or *interoceptive*". Establishing, extinguishing, and reversing a heterogeneous operant chain might further explicate different sets of relations established among an interoceptive drug condition, the specific responses that are evoked within the chain, and the primary reinforcer. Such evaluations might be clinically appealing in view of the theoretical points made earlier regarding extinction of interoceptive stimulus control over drug-seeking behavior. The present investigation therefore sought to attain, extinguish, and reverse conditional discriminative control by nicotine over two different response sequences using a heterogeneous operant chain consisting of two topographically distinct responses (i.e., a lever-press and a nose-poke). More specifically, the prediction that the order of responses within the sequence (nose-poke→lever-press vs. lever-press→nose-poke) could come under conditional discriminative control by nicotine was tested.

The study was carried out using a within-subjects design. Methodologically, nicotine was used as an interoceptive cue under which one sequence was reinforced by food pellet delivery, whereas the opposite sequence was reinforced under saline (non-drug). Specifically, on some sessions, the completion of the lever-press→nose-poke sequence was reinforced following pre-session administration of nicotine, whereas the nose-poke→lever-press sequence was reinforced on saline sessions. The distal response in both sequences was always maintained on a variable interval 30-sec (VI-30) schedule and its completion resulted in the presence of a stimulus light. When the light was present, the proximal response (maintained on a fixed ratio-1 schedule; FR-1) was followed by a food pellet delivery (S^{r+}). In view of the contrast between the schedule requirements for the distal (VI) and proximal (FR-1) responses, it was predicted that distal response rates would exceed proximal response rates during training sessions with food reinforcement but most importantly also during brief extinction tests conducted without food delivery or the presentation of the light. Such differences in response rates (schedule control) during these tests served as the dependent variable to evaluate the relationships learned among the nicotine and saline conditions and each of the two responses within the chain.

As noted earlier, extinction methods were used previously by this laboratory to evaluate relationships learned among the drug- S^D , the response, and the primary reinforcer. For

instance, Troisi (2003a) found that extinction training with saline did not disrupt the ability of nicotine to evoke discriminated responding. When nicotine was administered differential control over responding was preserved. Therefore, in a second phase of the present study, the nose-poke→lever-press sequence was first extinguished over ten sessions under saline. It was predicted that nose-poke rates (the distal response) would be greater than lever-press rates (the proximal response) and would decrease over the consecutive extinction sessions. Ten additional extinction sessions were conducted to evaluate the impact of nicotine on differential responding of the lever-press→nose-poke sequence. It was predicted that when nicotine was administered, lever-pressing rates would initially increase and be greater than nose-poking rates but would then decrease over the consecutive extinction sessions.

In view of the reversal learning data with nicotine previously reported (Troisi et al, 2010), in a third phase the stimulus roles of nicotine and saline were switched to evaluate reversal of conditional control by nicotine and saline over the two sequences. It was predicted that reassigning the stimulus roles of nicotine and saline over the two response sequences in the present investigation would reverse performance on each response manipulandum. Finally, to evaluate the potential stimulus function, the light was removed during the final reversal sessions. It was predicted that distal response rates would diminish.

Methods

Animals

Eight one-year old male Sprague-Dawley rats (Harlan Breeders, Indianapolis, IN) maintained at 80% of free-feeding weights (350-400gm) were used in accord with this institution's IACUC policies. Water was available at all non-experimental times. All animals were weighed daily in the morning and fed at approximately 5:30 pm Monday through Friday and at approximately 12:00 pm on weekends. One session took place on a Saturday (see below). Following this session the rats were fed no less than two hours following the completion of the session. Rats were housed individually in stainless steel cages measuring L 24.5 cm × W 20.0 cm × H 17.5 cm. Plexiglas liners were located above the mesh floor to contain floor bedding. A 12 hour light-dark cycle (0700-1900 hours) was maintained. The rats had equal reinforcement and extinction training histories involving lever-pressing and nose-poking in a behavioral resurgence investigation. Both responses in that investigation were maintained on a VI-30 sec schedule of food reinforcement and also underwent an equal number of sessions of extinction. This investigation was completed 3 month prior to the present investigation. During the intervening 3 months, the rats were maintained at 80% of their free-feeding weights and were not used experimentally for any purpose.

Apparatus

Sessions took place in eight stainless-steel operant chambers (Med-Associates, Georgia VT, model ENV-001) measuring L 28 × W 21 × H 21 cm. The chambers were placed two to three feet apart and located about the perimeter of the sound and light attenuated experimental room. The room measured L 16.5 × 9 feet. The chambers were not equipped with houselights. An antenna-less/cable-less television set delivered a white noise source that was initiated at the start of each session and terminated at the end of the session by the

investigator. Overhead recessed incandescent track lights controlled by a dimmer switch produced approximately 15 watt lighting during session time and was terminated at the end of each session by manually turning on the bright overhead florescent room-lighting. Each chamber was equipped with one lever located 2 cm to the left of the centrally-located food magazine (which delivered 45 mg food pellets, BioServe, Frenchtown, NJ) and 7 cm above the grid floor. A stimulus light, which functioned as the exteroceptive S^D, was located above the lever. Nose-poke devices (Med-Associates) were located 2 inches from the rear stainless steel wall on the adjacent Plexiglas wall to the left of the lever at the rear end of the chamber. Nose poke entry by the rat's snout triggered a photocell which was recorded by the Med-PC software version 2.0 via a PC computer in an adjacent room. Levers and nose poke devices were present during all sessions at all times.

Procedure

Procedural overview—The general procedure consisted of training, testing, and extinction, and reversal phases. During training, one sequence was established with nicotine and the opposite sequence was established with saline. On sessions 20 and 21 brief extinction tests were conducted without food reward. These two tests were followed by additional training sessions, which were followed by 20 consecutive extinction sessions. The first 10 were conducted with saline and the remaining 10 were conducted with nicotine. Reversal training then commenced in which the roles of nicotine and saline were switched for each of the response sequences. Finally, the light that linked the two responses was omitted during the final six sessions.

Drugs and drug administration—(–)-Nicotine di-tartrate (Research Biochemicals International, Natick, MA, USA) was dissolved in 0.9% NaCl saline and the dose (0.4 mg/kg) expressed as the base. This dose was selected because its discriminative stimulus effects are well documented in the drug discrimination literature (e.g., Stolerman et al. 1984) and has been used in prior reports from this laboratory. Animal weights were recorded just prior to drug preparations each day. The intraperitoneal (ip) injections (1 ml/kg) took place approximately ten minutes before the start of all drug discrimination training sessions.

General procedure and initial chain training—No injections took place on (or prior to) the first four sessions in which the operant chain was established. Sessions took place between 12:30 pm and 3:30 pm and were conducted predominantly Monday through Friday. One session was conducted on a Saturday. At the start of each session, the overhead florescent lights were turned off by the investigator and the white noise source was initiated. The experimental room was dimly lighted and there were no houselights illuminated in the chambers at any point in the study. The 30-min session was then initiated manually by computer operation. When the session was completed, the overhead lights were immediately turned on and the white noise source was terminated manually.

No specific training was necessary because of the rats' experimental histories established in the investigation completed three months earlier. Moreover, a backward chaining procedure, commonly used for establishing long operant chains with several responses and linking stimuli, was not necessary since both responses had been previously established. During the

first 4 sessions, the operant chain was acquired without injection of drug or saline. During these and all sessions the nose-poke devices and the levers were present and the rats were free to respond on either manipulanda at all times. On these first 4 sessions nose-poking was maintained on a VI-30 sec schedule and constituted the distal response. The VI schedule ranged from 10 sec to 50 sec by 5 sec increments, which were randomly selected upon each completion of the schedule by the RANDI function in the Med-PC. Completion of the VI schedule resulted in the illumination of the stimulus light. Lever-pressing (the proximal response) was then reinforced on a fixed ratio of one (FR-1) by a food pellet, which also terminated the light. There was no timeout following food delivery and the distal VI-30 sec response schedule was reinstated immediately. The proximal response was not reinforced unless the distal response schedule requirement had been completed and the light was present, but otherwise occurred freely and was recorded at all times. Similarly, responding on the distal response manipulanda did not result in food delivery when the schedule was completed (i.e., when the light was presented) but was recorded. This chain sequence is thus diagrammed nose→lever and was in effect in the saline condition described below. The average number of reinforcers earned per session was approximately 51.

Acquisition training—Table 1 shows the chain sequences that were in effect for the nicotine and saline conditions. Eight minutes following injections the rats were transported as a group in a Nalgene® container from their home cages to the conditioning chambers. On the first three sessions saline was administered to accustom the rats to the injection procedure; thus the nose→lever sequence was in effect. On nicotine sessions the order of the responses in the chain was reversed. Lever-pressing was maintained on the VI-30 sec schedule and resulted in the presentation of the light. When the light was presented, the first nose-poke resulted in food delivery. The sequence is diagrammed lever→nose. Again, both responses were free to occur throughout all sessions and were recorded at all times. Nicotine and saline conditions were randomly selected from day to day by a coin-flip under the constraint that no more than two consecutive presentations of either condition could occur (except during reversal). There were a total of 35 acquisition sessions and two brief non-reinforcement tests were conducted at the start of the 20th and 21st session (see next).

Brief extinction tests—At the outset of the 20th and 21st acquisition sessions brief 3-min extinction tests were conducted approximately 10 min following injections. During these two extinction tests no scheduled consequences were in effect for either response; that is, distal responses did not result in presentation of the light and proximal responses did not result in food delivery. Following these 3-min tests the usual programmed consequences were in effect for 30-min. It should be noted that only two brief tests were conducted to minimize the potential for response-suppression that might otherwise have occurred with repeated non-reinforcement tests throughout acquisition training.

Extinction sessions—During the first 10 sessions following acquisition, both responses were non-reinforced in the saline condition. During these sessions, nose-pokes (distal response) did not result in the presentation of the light and lever-presses (proximal response) did not result in food delivery. Over the next 10 sessions, nicotine was administered and again both responses were without consequence. It should be noted here that the rationale

for carrying out extinction of saline responding first with all eight rats was two-fold: First, as noted above, the rats had histories of acquisition and extinction in the non-drug state in a previous resurgence investigation. The concern was, that extended extinction sessions carried out first in the nicotine condition might compromise the potential recovery of the opposite response chain in the saline condition. Second, the authors sought to systematically replicate Troisi (2003a) results showing that extinction in the non-drug state does not subsequently undermine discriminative control by nicotine.

Reversal training—Over the next 30 sessions the roles of nicotine and saline were switched. One rat was removed during this phase because the stimulus light output interface bit failed due to a short circuit that occurred. The nicotine condition now occasioned the nose→lever sequence and saline occasioned the lever→nose sequence. Six additional sessions were conducted without the light. During these final sessions completion of the distal response VI schedule did not result in the light but did initiate the proximal response FR-1 schedule, which, when fulfilled, resulted in food pellet delivery.

Results

Acquisition training

Figure 1 displays the acquisition results. Mean responses ($\pm$ sem) per minute (averaged across the entire session) are presented for each response topography in each drug condition. Because specific predictions were made concerning differences between distal vs. proximal response rates, the rates of responding were averaged over all acquisition sessions and compared by dependent t-tests ($\alpha = .05$). The rates were averaged across all sessions as a more conservative average relative to a specified number of sessions. Rates of responding on the distal response manipulanda were significantly greater than on the proximal response manipulanda; on average, in the nicotine condition, the rate of lever pressing (dark squares; $M = 14.18$ responses/minute, $SEM = 1.79$) was significantly greater than nose poking (dark circles; $M = 6.21$ responses/minute, $SEM = .47$) [$t_{(7)} = 4.73$, $p = .002$]. Conversely, in the saline condition the rate of nose poking (clear circles; $M = 16.01$ responses/minute, $SEM = 2.92$) was significantly greater than lever pressing (clear squares; $M = 4.96$ responses/minute, $SEM = .81$) [$t_{(7)} = 3.65$, $p = .008$].

Responding on the proximal response manipulanda also occurred throughout the sessions during the distal response component of the sequences (i.e., before the light had been initiated). These responses (not shown) were without programmed consequences. They were obtained by subtracting FR-1 reinforced responses (i.e., those that occurred during the proximal schedule when the light was present) from the total responses emitted on the proximal response manipulanda. The rate of nose-poking ($M = 3.29$ responses/min; $SEM = .81$) during the distal component of the sequence in the nicotine condition did not differ significantly from the rate of lever pressing ($M = 4.73$ responses/min; $SEM = .54$) in the distal component of the sequence in the saline condition [$t_{(7)} = 2.04$, $p = .081$].

Brief extinction tests

The results of the two 3-min extinction tests that were conducted at the outset of sessions 20 and 21 are displayed in Figure 2. These data are consistent with the acquisition results. Response rates on the distal response manipulanda were greater than on the proximal response manipulanda. In the saline condition, rates of nose-poking were significantly greater than rates of lever-pressing [$t_{(7)} = 3.28, p = .013$]; conversely, in the nicotine condition, rates of lever-pressing were greater than rates of nose-poking [$t_{(7)} = 2.72, p = .03$].

Extinction sessions

Figure 3 displays the results of the extinction sessions. Nose-poke rates decreased significantly over the course of the ten extinction sessions as revealed by a repeated measures ANOVA [$F_{(1,7)} = 34.43, p = .001; r^2 = .98$] as did lever-press rates [$F_{(1,7)} = 35.61; p = .001; r^2 = .98$]. At the outset, response rates on the distal response manipulanda were greater than on the proximal response manipulanda. In the saline condition (Fig 3, left) nose poking rates were initially greater than lever pressing rates. Averaged over all ten extinction sessions, nose-poke rates were significantly greater than lever-press rates [$t_{(7)} = 3.27; p = .014$]. When nicotine was administered (Fig 3, right) lever-press rates increased significantly on the first session (session 11) compared to the previous session conducted in the saline condition [$t_{(7)} = 4.82; p = .002$], whereas nose-poke rates remained low. Over the course of the ten extinction sessions, lever-press rates decreased significantly in a linear manner [$F_{(1,7)} = 16.01; p = .005$] but nose-poke rates did not [$F_{(1,7)} = 1.47; p = .26$]. Averaged over all ten extinction sessions lever-press rates were significantly greater than nose-poke rates [$t_{(7)} = 3.45; p = .011$].

Reversal training and removal of the light

Figure 4 shows the reversal training results. Switching the roles of nicotine and saline affected response rates within both sequences. Averaged over all 30 sessions distal nose-poke rates ($M = 25.49$ responses/min; $SEM = 3.73$) were significantly greater than proximal lever-press rates ($M = 8.87$ responses/min; $SEM = 1.39$) in the nicotine condition [$t_{(6)} = 4.25; p = .005$]. By contrast, in the saline condition distal lever-press rates ($M = 13.53$ responses/min; $SEM = 1.64$) were only marginally different from proximal nose-poke rates ($M = 9.53$ responses/min; $SEM = 1.47$) [$t_{(6)} = 2.26, p = .065$].

Removal of the light significantly altered response rates in the nicotine and saline conditions (Fig 4, far right). Nose-poke and lever-press response rates were averaged separately across all three of the six sessions conducted within each of the two drug conditions and were compared to the rates that were averaged across the final 3 sessions in each condition conducted just prior to removal of the light. With nicotine, distal nose-poke rates decreased [$t_{(6)} = 4.19, p = .006$] whereas proximal lever-press rates increased [$t_{(6)} = 4.16, p = .006$]. In the saline condition, distal lever-press rates decreased [$t_{(6)} = 5.07, p = .002$] but there was no significant change in proximal nose-poke rates. Additionally, there was a significant difference between distal nose pokes and proximal lever press rates with nicotine [$t_{(6)} = 2.83, p = .03$]; interestingly, proximal rates now exceeded distal rates. There was no significant difference in the saline condition [$t_{(6)} = 1.92, p = .104$].

Discussion

To this investigator's knowledge, this is the first investigation to report that a heterogeneous operant chain involving two topographically distinct responses can come under conditional control by a drug state (i.e., nicotine). Nicotine occasioned a lever-press→nose-poke sequence and saline occasioned a nose-poke→lever-press sequence. Because of the contrast between the distal and proximal reinforcement schedules (VI-30 sec and FR-1, respectively) it was predicted that distal response rates (regardless of topography) would be greater than proximal response rates throughout the training sessions, brief extinction tests, and during the consecutive extinction sessions. These hypotheses were supported. Similar results were evident when the conditional roles of nicotine and saline were reversed for each sequence.

The results from the twenty consecutive extinction sessions add validity to the results of the brief 3-min extinction tests. As predicted, during the first 10 sessions in which saline was administered, nose-poke rates (the distal response previously maintained on VI-30) initially exceeded lever-press rates (the proximal response previously maintained on FR-1) but gradually diminished. Concomitantly, lever-pressing rates remained low throughout extinction training. As further predicted, when nicotine was subsequently administered, rates of lever-pressing (distal response) increased but nose-poke rates (proximal response) remained relatively low. Extinction of both responses then occurred. These results are consistent with a previous report (Troisi, 2003a) demonstrating that extinction of responding in the non-drug state (i.e., saline) does not undermine the original stimulus control established with nicotine. These data may suggest that extinction of the nose-poke→lever-press sequence under saline was state-dependent because such extinction did not *transfer* to the lever-press→nose-poke sequence when further extinction training took place with nicotine (e.g., Bouton, Kenney, & Rosengard, 1990). Thus, each response chain appeared to be under conditional control by nicotine and saline. A future investigation should counterbalance the order in which saline and nicotine were presented during extinction to better evaluate potential directionality in state-dependent control.

The light that linked the distal and proximal response schedules in the nicotine and the saline conditions appeared to play a role in mediating the response sequences. Removal of the light during the reversal training appeared to disrupt response rates, particularly of the distal response. It is possible that the light functioned as a *conditioned reinforcer* that maintained the distal response – and as a discriminative stimulus that signaled the proximal response in the sequence (Kelleher & Gollub, 1962). However, the limited sample size and lack of counterbalance across the nose-poke and lever press responses precludes clear evidence for this hypothesis. Furthermore, it is important to acknowledge that the light was located just above the lever and not the nose-poke device. It is possible that the light may have merely evoked a switching response from one response to another. Counterbalancing the roles of the response manipulanda may be critical for ruling out a response sequence preference. Ultimately, the interoceptive drug condition likely interacted with the role of the light in regulating the ongoing sequences of behavior. This interpretation is consistent with other studies that showed conditional discriminative relations among drug states and exteroceptive stimuli in both operant and Pavlovian drug discrimination procedures (Bevins & Murray, 2011; Colpaert, Niemegeers, & Janssen, 1978; Duncan, Phillips, Reints, & Schecter, 1979;

Jarbe and Johansson 1984; Jarbe, Sterner, & Hjerpe, 1981; Parker, Schaal, & Miller, 1994; Troisi & Akins, 2004).

Switching the conditional roles of nicotine and saline over the response sequences appeared to promote differing reversal acquisition profiles. Distal and proximal response rates of the nose-poke→lever-press sequence differed more greatly in the nicotine condition than those of the lever-press→nose-poke sequence in the saline condition. Why this reversal was not symmetric is not clear. One possibility is that nicotine facilitated learning of the reversal contingencies. Nicotine has been shown to influence attention and learning (Semenova, Stoleran, and Markou, 2007). Alternatively, the rats' reinforcement and extinction histories in the non-drug state with both responses in the pilot study conducted 3 months prior to this investigation may have contributed to weaker reversal learning in the saline condition. Nevertheless, it appeared that the conditional control by nicotine over the response sequences established in the initial acquisition phase was reversed when the response outcome contingencies were switched. These data are consistent with a recent investigation in which the conditional roles of nicotine and ⁹-THC were reversed for lever-pressing and nose-poking topographies (Troisi et al., 2010); they are also consistent with other evaluations of reversal learning of drug discriminations (e.g., Rijnders, Jarbe, & Slangen, 1991). The present study thus provides further evidence for the importance of the specific relationships learned between the drug-S^D and the response(s) it evokes in sustaining operant drug discrimination.

Finally, the current data may be clinically relevant. First, drug-seeking and drug-taking behavior(s) involve complex repertoires, which can be mediated by antecedent interoceptive stimuli (emotions, stress, thirst, hunger, or other drug states). A future investigation might combine the current drug discrimination method using drug-reinforcement rather than food-reinforcement (e.g., Beardsley *et al.* 1992). Second, in view of the literature showing that nicotine increases reinforcing effects of exteroceptive stimuli (e.g., Palmatier, Liu, Matteson, Donny, Caggiula, Sved, 2007) and the ramifications for smoking rituals and tobacco addiction, the methods presented here governing complex sequences of behavior may be applicable. To be sure, nicotine regulated the response chains in the present investigation and the light linking the responses appeared to be important in this regard. Third, *cue exposure therapy*, based on Pavlovian extinction of conditioned responding (e.g., craving) to exteroceptive drug-associated stimuli (drug paraphernalia) has been unsuccessful (Conklin & Tiffany, 2002). Conklin & Tiffany noted that extinction of the operant self-administration response in the presence of operant cues that occasion drug availability may be more clinically effective than merely presenting Pavlovian cues. However, it may be equally important to extinguish behavior that is under the control of interoceptive discriminative control (e.g., emotions, stressors, or even other drug states) over drug-seeking and drug-taking rituals that have contingent relations with drug effects in specific exteroceptive contexts, rather than merely carry out Pavlovian extinction (cf., Bevins & Murray, 2011). The operant drug discrimination paradigm may be useful in simulating extinction of interoceptive control over behavior (see Troisi, Bryant, & Kane, 2012). In the present study, extinction of one response sequence in a particular interoceptive state did not appear to greatly undermine stimulus control over a different response sequence associated

with the same reinforcing outcome (food) that was previously established in a different interoceptive state. Clinically, it may be important to extinguish multiple drug-taking rituals (smoked vs. IV heroin administration) in multiple interoceptive states with similar exteroceptive S^D's in contexts that were maintained by the same drug-reinforcer. To conclude, here it was shown that nicotine established conditional control in modulating different sequences of food-seeking behavior in rats that were likely mediated by an exteroceptive operant conditioned reinforcer.

Acknowledgments

This work was supported by New Hampshire IDeA Network of Biological Research Excellence (NH-INBRE) NIH Grant Number 1P20RR030360-01 from the INBRE Program of the National Center for Research Resources. I am indebted to Drs. Torbjörn (Toby) U. C. Järbe of the Center for Drug Discovery at Northeastern University, Boston MA, Ian P. Stolerman of the Institute of Psychiatry, King's College, London, England, and Leigh Panlillio of NIDA for reading and commenting various versions of this manuscript. Special thanks go to Lauren Morse & Erin Bryant for data collection and data entry, and Dustin MacConnel & Donna Pioli for their diligent animal husbandry.

References

- Beardsley PM, Anthony EW, Lopez OT. Phencyclidine established as a discriminative stimulus using ethanol as a reinforcer. *Behavioural Pharmacology*. 1992; 3:497–505.10.1097/00008877-199210000-00008 [PubMed: 11224152]
- Bevins, RA.; Murray, JE. Internal stimuli generated by abused substances: role of Pavlovian conditioning and its implications for drug addiction. In: Schachtman, TR.; Reilly, S., editors. *Associative Learning and Conditioning: Human and Non-Human Applications*. Oxford University Press; New York: 2011. p. 270-289.
- Bouton ME, Kenney FA, Rosengard C. State-dependent fear extinction with two benzodiazepine tranquilizers. *Behavioral Neuroscience*. 1990; 104:44–55.10.1037/0735-7044.104.1.44
- Colpaert FC, Koek W. Empirical evidence that the state dependence and drug discrimination paradigms can generate different outcomes. *Psychopharmacology*. 1995; 120(3):272–279.10.1007/BF02311174 [PubMed: 8524974]
- Colpaert FC, Niemegeers CJE, Janssen PAJ. Drug-cue conditioning to external stimulus conditions. *European Journal of Pharmacology*. 1978; 49:185–188. [http://dx.doi.org/10.1016/0014-2999\(78\)90076-6](http://dx.doi.org/10.1016/0014-2999(78)90076-6). [PubMed: 658134]
- Conklin CA, Tiffany ST. Applying extinction research and theory to cue-exposure addiction treatments. *Addiction*. 2002; 97:155–167.10.1046/j.1360-0443.2002.00014.x [PubMed: 11860387]
- D'Andrea T. Extinction of a heterogeneous chain after several reinforcement schedules. *Journal of the Experimental Analysis of Behavior*. 1969; 12:127–135. [PubMed: 16811333]
- Duncan PM, Phillips J, Reints J, Schechter MD. Interaction between discrimination of drug states and external stimuli. *Psychopharmacology*. 1979; 61:105–106.10.1007/BF00426820 [PubMed: 108713]
- Everitt BJ, Robbins TW. Second-order schedules of drug reinforcement in rats and monkeys: Measurement of reinforcing efficacy and drug-seeking behaviour. *Psychopharmacology*. 2000; 153(1):17–30.10.1007/s002130000566 [PubMed: 11255926]
- Järbe TU, Johansson B. Interaction between drug discriminative stimuli and exteroceptive, sensory signals. *Behavioral Neuroscience*. 1984; 98(4):686–694.10.1037/0735-7044.98.4.686 [PubMed: 6466444]
- Järbe TUC, Sterner U, Hjerpe C. Conditioning of an interoceptive drug stimulus to different exteroceptive contexts. *Psychopharmacology*. 1981; 73:23–26.10.1007/BF00431094 [PubMed: 6785784]
- Kelleher RT, Gollub LR. A review of positive conditioned reinforcement. *Journal of the Experimental Analysis of Behavior*. 1962; 5:543–597.10.1901/jeab.1962.5-s543 [PubMed: 14031747]

- Lattal KA, Crawford-Godbey CL. Homogeneous chains, heterogeneous chains, and delay of reinforcement. *Journal of the Experimental Analysis of Behavior*. 1985; 44:337–342.10.1901/jeab.1985.44-337 [PubMed: 16812436]
- Lu B, Li M, Hou Y-y, Chen J, Chi Z-q, Liu J-g. Initial experience of heroin use under a two-chained operant schedule influences drug-seeking behavior one month of abstinence. *Acta Pharmacologia Sinica*. 2010; 31:387–392.10.1038/aps.2010.16
- Palmatier MI, Liu X, Matteson GL, Donny EC, Caggiula AR, Sved AF. Conditioned reinforcement in rats established with self-administered nicotine and enhanced by noncontingent nicotine. *Psychopharmacology*. 2007; 195:235–243.10.1007/s00213-007-0897-6 [PubMed: 17676401]
- Panlilio LV, Thorndike EB, Schindler CW. A stimulus-control account of regulated drug intake in rats. *Psychopharmacology*. 2008; 196:441–450.10.1007/s00213-007-0978-6 [PubMed: 17957355]
- Parker BK, Schaal DW, Miller M. Drug discrimination using a Pavlovian conditional discrimination paradigm in pigeons. *Pharmacology Biochemistry and Behavior*. 1994; 49:955–960. [http://dx.doi.org/10.1016/0091-3057\(94\)90249-6](http://dx.doi.org/10.1016/0091-3057(94)90249-6).
- Pelloux Y, Everitt BJ, Dickinson A. Compulsive drug seeking by rats under punishment: effects of drug taking history. *Psychopharmacology*. 2007; 194:127–137.10.1007/s00213-007-0805-0 [PubMed: 17514480]
- Pierrel R, Sherman JG. Barnabus, the rat with college training. *Brown Alumni Monthly*. 1963 Feb.:8–14.
- Rijnders HJ, Järbe TUC, Slangen JL. Reversal of a drug versus drug discrimination task with different exteroceptive conditions. *Behavioral Pharmacology*. 1991; 2:429–435.10.1097/00008877-199109000-00018
- Semenova S, Stolerman IP, Markou A. Chronic nicotine administration improves attention while nicotine withdrawal induces performance deficits in the 5-choice serial reaction time task in rats. *Pharmacology Biochemistry and Behavior*. 2007; 87:360–368.10.1016/j.pbb.2007.05.009
- Shindler CW, Panlilio LV, Goldberg SR. Second-order schedules of drug self-administration in animals. *Psychopharmacology*. 2002; 163:327–344.10.1007/s00213-002-1157-4 [PubMed: 12373434]
- Siegel S. Drug tolerance, drug addiction, and drug self-administration. *Current Directions in Psychological Science*. 2005; 14:296–300.10.1111/j.0963-7214.2005.00384.x
- Stolerman IP, Garcha HS, Pratt JA, Kumar RR. Role of training dose in discrimination of nicotine and related compounds by rats. *Psychopharmacology*. 1984; 84:413–419.10.1007/BF00555223 [PubMed: 6440189]
- Troisi JR II. Nicotine vs. ethanol discrimination: Extinction and spontaneous recovery of responding. *Integrative Physiological & Behavioral Science*. 2003a; 38(2):104–123.10.1007/BF02688829 [PubMed: 14527180]
- Troisi JR II. Spontaneous recovery during, but not following, extinction of the discriminative stimulus effects of nicotine in rats: Reinstatement of stimulus control. *The Psychological Record*. 2003b; 53:579–592.
- Troisi JR II. Pavlovian-instrumental transfer of the discriminative stimulus effects of nicotine and ethanol in rats. *The Psychological Record*. 2006; 56(4):499–512.
- Troisi JR II. Pavlovian extinction of the discriminative stimulus effects of nicotine and ethanol in rats varies as a function of context. *The Psychological Record*. 2011; 61(2):199–212.
- Troisi JR II. Perhaps more consideration of Pavlovian-operant Interaction may improve the clinical efficacy of behaviorally based drug treatment programs. *The Psychological Record*. in press.
- Troisi JR II, Akins C. The discriminative stimulus effects of cocaine in a pavlovian sexual approach paradigm in male Japanese quail. *Experimental and clinical psychopharmacology*. 2004; 12(4): 237. [PubMed: 15571440]
- Troisi JR II, Bryant E, Kane J. Extinction of the discriminative stimulus effects of nicotine with a devalued reinforcer: Recovery following revaluation. *The Psychological Record*. 2012; 62(4):707–718.
- Troisi JR, LeMay BJ, Järbe TU. Transfer of the discriminative stimulus effects of 9-THC and nicotine from one operant response to another in rats. *Psychopharmacology*. 2010; 212(2):171–179. [PubMed: 20628732]

Fig 1.

Acquisition of a conditional discrimination involving a heterogeneous chain. Mean responses per minute ($\pm$ sem) during each of 35 training sessions of an operant heterogeneous chain for eight rats. The first four sessions (left) were conducted without injection. Under nicotine (0.4 mg/kg, ip) administration (dark symbols) lever-pressing on VI-30 sec schedule (distal response) resulted in illumination of a stimulus light- S^D , which occasioned nose-poking (proximal response) maintained by food delivery on an FR-1. Under saline (clear symbols) the order of the responses in the sequence chain was reversed; nose-poking (distal) on a VI-30" resulted in the illumination of the stimulus light, which occasioned lever-pressing (proximal) maintained by food delivery on an FR-1. Nicotine and saline conditions were random from day to day under the constraint of no more than two consecutive presentations of either condition. "Distal" indicates that the response was distal in the chain and "Proximal" indicates proximal in the response-sequence chain. Down arrows demarcate sessions in which brief 3-min non-reinforcement tests were conducted (sessions 20 and 21).

Fig 2.

Mean responses per min ($\pm$ sem) during two 3-min pre-session extinction tests conducted at the outset of sessions 20 and 21. Both responses were without consequence. The sequence training histories are labeled under each set of bars (distal→proximal) for saline (left bars) and nicotine (right bars).

Fig 3.

Twenty 30-min extinction sessions carried out with saline (left, clear symbols) and with nicotine (right). The response sequences that were operative during training are posted at the top (distal→proximal).

Fig 4.

Conditional reversal training of a heterogeneous operant chain. Mean rate of responding per min ($\pm$ sem) during each 30 min training session of each response in the chain. On nicotine sessions (dark symbols) nose poking was maintained by presentation of a light-S^D on a VI-30 sec and lever pressing was reinforced by food delivery on an FR-1. In the saline condition (clear) the sequence was the opposite. During the final six sessions, the light-S^D was removed from the sequence but responding initiated the scheduled consequences.

Table 1

Drug Conditions and Response Sequences

<u>Condition</u>	<u>Distal Response</u>	<u>S^D</u>	<u>Proximal Response</u>	<u>S⁺</u>
Saline:	Nose (VI-30) →	Light:	Lever (FR-1) →	pellet
Nicotine:	Lever (VI-30) →	Light:	Nose (FR-1) →	pellet

Drug conditions that occasioned a heterogeneous operant chain that was conditional by the order of the response topographies that defined distal and proximal responses. Eight rats were exposed to the nicotine and saline conditions over the course of 35 acquisition sessions.