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Can the discriminative stimulus effects of nicotine function concurrently as modulatory opponents in operant and pavlovian occasion setting paradigms in rats?

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ABSTRACT

Nicotine promotes interoceptive changes in the nervous system. Such interoceptive stimuli play important roles in modulating addictive behavior. Operant and Pavlovian stimulus control modulate responsiveness to environmental stimuli related to drug-seeking and self-administration. Nicotine functions as a discriminative stimulus in modulating operant behavior as well as Pavlovian feature stimuli in modulating the conditional responding (CR) to exteroceptive CS→US contingencies. Elucidation of the interaction of these interoceptive stimulus control functions is vital for a comprehensive understanding of nicotine use/abuse, which might lead to better behavioral treatment strategies. This experiment evaluated the interaction among Pavlovian feature positive (FP) and feature negative (FN) effects of nicotine on concurrently occurring operant S^D and S^Δ effects. Sixteen rats were trained in a Pavlovian and operant bidirectional contingency paradigm, using nicotine (0.3 mg/kg) and non-drug (saline) states as interoceptive cues for operant discriminative stimulus conditions (S^D and S^Δ) as well as Pavlovian FP and FN for a light-CS, either leading to a shared food pellet outcome or non-outcome. Nicotine and saline sessions were intermixed. For one group of rats (n = 8), nicotine served as an S^D for lever pressing (variable interval 60 s) and simultaneously functioned as an FN for CS-light→noUS relation on the same sessions. On intermixed sessions, saline served as the S^Δ for lever pressing (non-reinforced) and FP, during which the 8-sec light preceded delivery of the food pellet (variable time ITI = 60 s). For the other group (n = 8) nicotine served as the S^Δ (lever pressing non-reinforced) and FP for the CS, with saline serving in the reverse roles. Consecutive brief non-reinforcement tests revealed that: A) rates of lever pressing were significantly greater in S^D than S^Δ with nicotine and saline suggesting strong operant discriminative stimulus control; B) FP responding to the light CS with nicotine and saline was evident; and C) FN suppression of the CR with nicotine was not evident but weak under saline. These data suggest that nicotine can function as an interoceptive context that hierarchically can enter into concurrently opposing modulatory relations in Pavlovian and operant drug discrimination procedures.

1. Introduction

Nicotine poses an international health threat because of its carcinogenic properties. In humans, it promotes a host of interoceptive changes ("subjective effects") including alertness, increased mood, and a subtle "high" (Benowitz, 1988). Nicotine also modulates responsiveness to exteroceptive stimuli predictive of drug-reward (see Hogarth and Troisi, 2015; Troisi, 2003a,b; Troisi, 2014). For instance, nicotine increases the conditioned reinforcing properties of exteroceptive stimuli with which it is associated (e.g., Olausson et al., 2004). Interestingly, whereas exposure to ethanol increases tobacco smoking (e.g., Mintz et al., 1985), nicotine exposure increases alcohol self-administration in humans (Barret et al., 2006). Thus, interoceptive drug states modulate responding to exteroceptive cues predictive of drug reward.

Identification of the antecedent stimulus control functions that modulate drug seeking and drug self-administration is important for understanding and treating nicotine addiction (Hogarth and Troisi, 2015). Indeed, Pavlovian stimulus control plays a contributive role in governing drug self-administration (e.g., Everitt et al., 2018). However, drug taking is operant behavior and is sensitive to its reinforcing consequences. Accordingly, it is equally critical to evaluate antecedent stimulus control that *sets the occasion* for actions leading to primary reward (Hogarth and Troisi, 2015; Troisi, 2013a,b,c; Troisi and Mauro,

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2017). Ultimately, Pavlovian and operant stimulus control interact in modulating drug-seeking behavior and self-administration. That the interoceptive stimulus effects of nicotine modulate smoking behavior, and increase responsiveness to cues associated with nicotine reward (or other drugs), suggests that Pavlovian and operant stimulus effects are likely to co-occur in this process. Evaluating the dynamics of Pavlovian-operant stimulus control interaction may contribute to a better understanding of nicotine addiction and its treatment (Troisi, 2014). Interoceptive stimulus control by nicotine with an appetitive outcome in concomitant Pavlovian and operant drug discrimination procedures is the focus of the present investigation. More specifically, the interaction between Pavlovian and operant stimulus control functions of nicotine was investigated. As noted elsewhere (e.g., Troisi, 2013c; Troisi and Craig, 2015) drug discrimination methodology may be useful for simulating the manner in which other interoceptive states (emotion, stress, hunger, sexual arousal, etc) modulate drug seeking and drug taking behavior. Our lab has employed nicotine as an interoceptive cue because it is readily discriminable and poses a health risk.

Nicotine functions as an interoceptive operant discriminative stimulus (i.e., S^D) that *sets the occasion* (Skinner, 1938) for voluntary response→ reinforcer relations in rats, or as an operant S^Δ in occasioning non-reinforcement of the response (Troisi, 2003a,b; Troisi, 2006; Troisi et al., 2013) – hence, *drug discrimination*. Conventionally, drug discrimination methodology has been employed to evaluate interoceptive (i.e., "subjective") effects of drugs in organisms that likely contribute to their reinforcing/rewarding properties and consequent abuse in humans. In order to evaluate traditional associative phenomena evident with the discriminative stimulus properties of nicotine, our lab uses a counterbalanced *go/no-go* (across session) drug discrimination procedure. For some rats, nicotine (0.3–0.4 mg/kg) is administered 10 min prior to sessions in which lever pressing (or nose poking) is food-reinforced on a variable interval (VI) schedule. Under this arrangement nicotine reliably functions as the S^D (i.e., go); whereas, saline (or an alternative drug state; e.g., alcohol) functions as the S^Δ occasioning intermixed sessions of non-reinforcement (i.e., no-go) (e.g., Troisi, 2003a; Troisi et al., 2010). For purposes of counterbalancing to control for potential unconditioned effects of the drugs on performance, nicotine's stimulus role is reversed for other rats and functions as S^Δ, whereas saline (or an alternate drug) functions as the S^D. This procedure also allows one drug condition to be explicitly paired with food-reward outcome (via the operant) and the alternate drug (or non-drug) to be explicitly unpaired with that outcome. Robust discriminative control develops within 18–24 sessions (c.f., Troisi, 2013a). Brief non-reinforcement tests conducted with each drug stimulus condition validates discriminative stimulus control: 1) S^D promotes significantly higher rates of responding than S^Δ; and 2) the discrimination indices for the S^D drug are greater than 80%, whereas those for the S^Δ drug are 20% or less.

In addition to its unconditioned stimulus (US) and conditioned stimulus (CS) effects (see Besheer et al., 2004), nicotine modulates exteroceptive Pavlovian CS→US relations as a *feature positive occasion setter* (FP) (Palmatier and Bevins, 2008); conversely, it also functions as a *feature negative occasion setter* (FN) stimulus signaling a CS→ noUS relationship. In that work, nicotine and saline sessions are intermixed. On sessions in which administration of nicotine serves as an FP, 15-s CS light presentations (with a variable time 2.5 min ITI) are followed by liquid sucrose US; whereas, on saline sessions the CS light presentations are not followed by the US, i.e., saline is the FN condition. Dipper entry (the conditional response) during the CS light is facilitated when nicotine functions as an FP occasion-setting state, and less responding to the CS is manifested for those rats under the non-drug saline state. With the FN work, the stimulus contingencies are reversed. On nicotine sessions, the CS does not follow the US, and on saline sessions, it does follow the US. With this arrangement, dipper entry during the CS is suppressed under nicotine relative to saline, and shows retarded reacquisition of excitation (Murray et al., 2011; c.f., Troisi and Akins, 2004). Although FP and FN effects have been reported in separate investigations, they are not used methodologically to counterbalance stimulus roles in individual investigations (see Troisi, 2013a; Troisi and Akins, 2004).

More theoretically, operant S^D/S^Δ and Pavlovian FP/FN occasion-setting stimulus contingencies have been proposed to share common hierarchical associative functions, as reported in the exteroceptive conditioning literature (Colwill and Rescorla, 1986; Rescorla, 1985). The S^D modulates the operant response-reinforcer relations, whereas the FP modulates Pavlovian stimulus-reinforcer relations. For instance, in transfer studies, operant S^Ds that set the occasion for lever pressing can subsequently *facilitate* the CR to a separately established appetitive CS. Similarly, an FP stimulus can subsequently function as an operant S^D that evokes operant responding (Davidson et al., 1988; c.f., Bonardi, 1989). In view of this, therefore, it might be informative to evaluate the extent to which both nicotine stimulus contingencies can co-occur in opposing modulatory roles concurrently in Pavlovian and operant stimulus arrangements. With opposing roles, interaction among the contingencies may be evident. If S^D/FP and S^Δ/FN share hierarchical relations, one role may compete with the other role when combined in opposition (e.g., Rescorla, 1987) (i.e., S^D vs. FN and S^Δ vs. FP). Therefore, the question becomes, can nicotine function as an operant S^D that sets the occasion for a lever-press→ food relation and simultaneously function within the same session (within subjects) as a Pavlovian FN stimulus that occasions the absence of a light-CS→ food-US relation? Conversely, can nicotine simultaneously function as an operant S^Δ that occasions the absence of a lever-press→ food relation and as a Pavlovian FP stimulus that sets the occasion for a light-CS→ food-US relation? Troisi (2006) demonstrated that nicotine, when first established as a CS+ or CS−, modulates operant responding associated with a common food reward – i.e., Pavlovian-instrumental transfer. The evaluation of interoceptive stimulus control by nicotine over co-occurring operant and Pavlovian contingencies might elucidate the nature in which interoceptive stimuli interact to elicit involuntary activity while also affecting voluntary response emission. The ramifications may be import for understanding the complexity by which interoceptive stimulus control modulates goal directed behavior for natural reward and drug reward. These questions were addressed in the present investigation using drug discrimination methods described earlier.

2. Materials and methods

2.1. Animals

Due to limited resources, 16 9-month old male Sprague Dawley rats (Harlan, Indianapolis) were maintained at 80% of their free-feeding weights (~350–400 g). They served in a prior pilot study in which nicotine and saline was administered at different times of the day to evaluate circadian effects on nose-poking behavior (see discussion section). Water was available at all non-experimental times. All animals were weighed daily in the morning and fed at approximately 5:30 p.m. Monday through Friday and at approximately 12:00 p.m. on weekends. Rats were housed individually in stainless steel hanging cages measuring L 24.5 cm × W 20.0 cm × H 17.5 cm. Plexiglas liners were located above the mesh floor to contain bedding. A 12 h light-dark cycle (0700–1900 h, light phase) was maintained. All animals were used in accord with Saint Anselm College's IACUC policies.

2.2. Apparatus

Sessions took place in eight stainless-steel operant chambers (Med-Associates, Georgia VT, model ENV-001) with a gridded floor measuring L 28 × W 21 × H 21 cm. The chambers were placed two to three feet apart and located around the perimeter of the sound and light attenuated experimental room that is designed for undergraduate courses in Learning & Conditioning, and Behavioral Pharmacology. The room

measured L 16.5 × 9 ft. 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 manually. Overhead recessed incandescent track lights controlled by a dimmer switch produced approximately 15 W lighting during session time and was terminated at the end of each session by manually turning on the bright overhead fluorescent lighting. Each operant chamber was equipped with one lever located 2 cm to the left of the centrally-located food magazine (which delivered standard 45 mg food pellets, BioServe, Frenchtown, NJ) and 7 cm above the grid floor. A stimulus light, which functioned as the CS target, was located directly above the lever. On Pavlovian test sessions an infrared video camera (Sony, Digital Handycam, cassette recorder – 120×) was placed on a tripod approximately 2 feet from the side door but focused on the food magazine and lever.

2.3. Procedure

2.3.1. Initial training

Daily sessions were generally run 5 days a week (with exception of college holidays, but occasionally on a Saturday or Sunday) as conducted in prior work by this lab since 2003 and others that study stimulus properties of drugs (e.g., see Troisi et al., 2010; work conducted at Northeastern University). Tests never occurred following a day off and always occurred following training sessions (see below). Sessions took place between 2:00 and 4:00 p.m. On the first day, lever pressing was acquired in all 16 rats by placing 5 food pellets on the lever at the start of training. Little training by the *method of successive approximations* was required, and all rats were lever-pressing reliably on an FR-1 for 30 min. On the second day, the schedule of reinforcement was shifted to a VI-30" and on the third day it was shifted to a VI-60". Sessions were 20-min. Three Pavlovian sessions intervened, during which the levers were removed, but 8-sec stimulus light presentations were paired with food pellet delivery. The average inter trial interval (ITI) was a variable time 60 s (VT-60").

2.3.2. Drug discrimination training

Drug discrimination training took place over the next 34 20-min sessions, 17 sessions with nicotine and 17 with saline. The rationale for conducting 34 sessions was because in our past work 18–24 sessions promoted robust stimulus control with nicotine; however, because of prior exposure to nicotine and saline, it was decided that an additional 10 sessions be conducted. Nicotine and saline sessions were intermixed, but no more than two consecutive sessions of either condition occurred and there had to be an equal number of reinforcement and non-reinforcement sessions. On nicotine days, rats received intraperitoneal injections of 0.3 mg/kg nicotine (calculated as base, dissolved in a 10% 10-X phosphate buffered saline vehicle, which maintained a constant pH of 7.0). This dose was selected because it is reliably promotes discriminative stimulus effects in this lab and others (Gauvin and Holloway, 1993; Troisi et al., 2010). On non-drug days, rats received intraperitoneal injections of the saline vehicle. The solution volume was 1.0 ml/kg for nicotine and saline administrations. 10 min following the injections, the rats were transported together as a squad of 8 in a white *Nalgene*® container from their home cages, down a short hallway, to the conditioning lab (described above) located in an adjacent room in the Animal Suite. Two squads of eight rats were run per day. In between running the squads, the chambers, levers, and food magazines were wiped with 10% EtOH to eliminate odor cues that could potentially bias responding (see Extance and Goudie, 1981). Each rat (Rd 1–8; Bu 1–8) was placed in the respective operant chamber (1–8). The fluorescent lights were then turned off, white noise was initiated, and the conditioning room doors were closed. The researcher exited the room and initiated the session manually on the computer in an adjacent room. When the 20 min session ended, the room light was illuminated and the white noise source was terminated, marking the end of the session.

For eight rats (group nicotine S^D/FN) nicotine functioned as S^D and lever pressing was reinforced on a VI-60" schedule. During these sessions, 8-sec CS-light presentations (ITI = VT-60", which was run on a separate timer) were not followed by food presentation, thereby allowing nicotine to also function as a FN state for the CS-light target. However, if the VI-60" timer for lever-pressing timed out, a lever-press during the 8-sec CS could produce a food pellet. For these same rats, on saline sessions, lever pressing was without consequence (i.e., saline functioned as S^Δ), but the 8-sec CS-light presentations were followed by food pellet US (i.e., saline also functioned as FP for the CS). For the remaining 8 rats (group nicotine S^Δ/FP), nicotine functioned as S^Δ and lever pressing was without consequence, but the 8-sec CS-light was followed by the pellet US, thereby allowing nicotine to also function as a FP for the CS-light target. On saline sessions, the contingencies were reversed; saline functioned as S^D for lever pressing and FN for the CS light target. Again, if the VI timer for lever pressing timed out, a lever-press could produce a pellet during the CS light. Total lever-presses per 20 min session were recorded.

2.3.3. Test sessions

There were two initial operant stimulus control tests, one conducted following the 16th training session and the other conducted after the 17th training session. One 3-min test was with nicotine and the other with saline counterbalanced by order and S^D/S^Δ stimulus roles across rats and across test days. During these two 3-min non-reinforcement sessions, the CS-light was never presented and lever pressing was without consequence under both drug conditions. Training sessions resumed as described above.

A second set of tests was conducted following the final training sessions, during which both Pavlovian and operant stimulus control were evaluated concurrently. During these tests, the levers were present, and the infrared video camera recorded behavior throughout the session. On these two test days, all 16 rats were tested individually because there was only one camera. Half the rats received nicotine on the first day and saline on the second day; for the remaining rats, the drug orders were reversed. During each session, there were 3 presentations of the CS-light, but without food-US. Lever pressing could occur freely but was without consequence. These test sessions had a duration range from 2.5 to 5.5 min. The session length varied from rat to rat because at least 3 presentations of the light occurred with an average ITI of VT-1 min. Total magazine entry rates (responses/min) during the CS-light and ITI were derived from the observer's evaluations. Observers were blind to the stimulus condition roles during those tests. Average ratings across all observers were used for data analysis. For example, if there was a mean total of 4 responses across all three CS presentations, the mean rate of magazine entry/min was 30.00 [(4/8 s)*60 s]. For ITI rates, total magazine entries were tabulated that occurred throughout all three ITIs; 24 s was subtracted from the test session length (which was converted from min to sec); thus, *x* responses divided by total ITI time in sec * 60 obtained the mean ITI rates. Lever press/min rates were derived by dividing total lever presses that occurred during the test session by the test session length that ranged from 2.5 to 5.5 min. These rates were compared across nicotine and saline sessions to determine operant stimulus control.

3. Results

3.1. Training data

The training results are displayed in Fig. 1. As evident, there was greater responding in the S^D conditions compared to the S^Δ conditions for both groups. Nicotine appeared to function equally well as S^D (top graph) and as S^Δ (bottom graph).

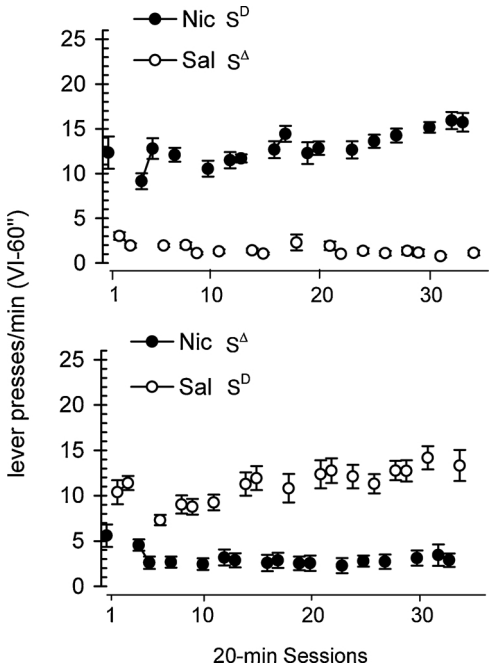

Fig. 1. Mean rate of lever pressing during drug discrimination acquisition training in which nicotine (0.3 mg/kg) functioned as an S^D in signaling food reinforcement on a VI-60" schedule during 20 min sessions, whereas saline functioned as S^Δ in signaling sessions of non-reinforcement for 8 rats (top graph). During those nicotine sessions, 8-sec light presentations were not followed by food pellets thereby assuming a Pavlovian feature negative role; during saline sessions the 8-sec light was followed by food allowing saline to function as a feature positive (FP). The roles of the drug conditions were counterbalanced for the data shown for the other 8 rats (bottom graph) in which nicotine functioned as the S^Δ and saline functioned as S^D; FP and FN roles of the drugs were reversed for the CS-light.

3.2. Test data

3.2.1. Initial operant stimulus control tests

Fig. 2 illustrates the results of the initial tests conducted on lever pressing rates under nicotine and saline that took place following the 16th training session. A 2 (groups: nicotine S^D/FN vs. nicotine S^Δ/FP) X 2 (drug conditions: nicotine vs. saline) repeated measure ANOVA ($\alpha = 0.05$) was conducted revealing no significant difference for group or drug condition, but a significant group by drug condition interaction [$F(1,14) = 105.3$; $p < .001$; $\eta^2 = 0.84$]. Paired-samples within group *t*-tests were conducted for each group. For the group assigned to the nicotine S^D/FN (left bars), there was significantly greater responding under nicotine S^D compared to the saline S^Δ [$t(7) = 7.73$; $p < .001$]. Similarly for the group assigned to the nicotine S^Δ/FP (right bars), there was significantly less responding under the nicotine S^Δ compared to the saline S^D [$t(7) = 6.81$; $p < .001$]. Discrimination indices [(total S^D responses/ total S^D + S^Δ responses) * 100] for the nicotine S^D averaged 86.3% (SEM = 1.05) and those for the nicotine S^Δ averaged 17.6% (SEM = .95), which is consistent with our prior investigations. Nicotine was as effective as an S^D in facilitating responding as it was an S^Δ suppressing responding.

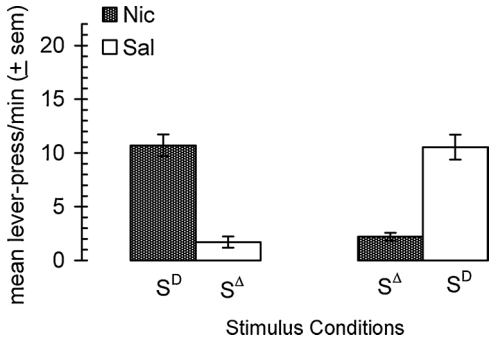

Fig. 2. Mean rate of lever pressing during two non-reinforcement tests conducted under nicotine and saline. The bars shown on the left display the results for the 8 rats that were trained with the nicotine S^D and saline S^Δ; the bars to the right show the results for the 8 rats in the counterbalanced group in which the stimulus roles of nicotine and saline were reversed.

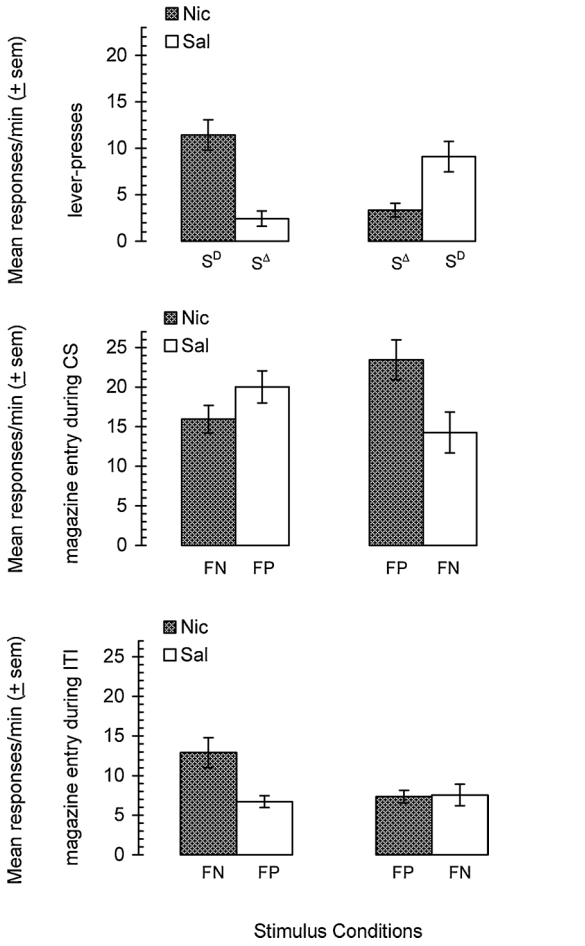

Fig. 3. Nonreinforcement test results for concurrently occurring discriminative stimulus effects of nicotine and Pavlovian goal tracking to the 8-sec light CS for the two groups trained with the nicotine S^D/FN (left bars) and nicotine S^Δ/FP (right graphs). During consecutive sessions of nicotine and saline, there were three presentations of the CS. Magazine entry rates are displayed under nicotine and saline. Lever pressing is shown to nicotine and saline (top graph). Goal tracking to the light is shown in the middle graph; and ITI rates are shown in the bottom graph.

3.2.2. Concurrent tests for Pavlovian FP / FN and operant S^D/S^Δ stimulus control

3.2.2.1. Operant stimulus control. Fig. 3 (top graph) displays the results for lever pressing under the S^D and S^Δ conditions for both groups. There were strikingly similar results compared to those obtained earlier in training (shown in Fig. 2). A 2 (groups) by 2 (drug conditions) repeated measures ANOVA revealed no significant effect for group or drug condition, but a significant group by drug condition interaction [$F(1,14) = 51.24$; $p < .001$; $\eta^2 = 0.76$]. For the group assigned to the nicotine S^D/FN (left bars) there was significantly greater responding under the nicotine S^D compared to the saline S^Δ as revealed by the paired-samples, within group *t*-test [$t(7) = 5.19$; $p = .001$]. Conversely, for the group assigned to the nicotine S^Δ/FP (right bars) there was a

significantly lower rate of responding compared to the saline S^D [t(7) = 5.15; p = .001].

3.2.2.2. Pavlovian feature stimulus control over the CS. Fig. 3 (middle graph) illustrates the results for magazine entry rates during the CS for both groups under nicotine and saline. A 2 (groups, nicotine S^D/FN vs. S^Δ/FP) X 2 (drug conditions) repeated measures ANOVA revealed no significant difference for group or drug condition, but a significant group X drug condition interaction [F(1,14) = 8.66; p = .011; η^2 = 0.38]. Magazine entry rates to the CS-light target under the nicotine FN condition were not significantly lower than the saline FP condition [t(7) = 1.34; p = .22] for the group assigned to the nicotine S^D/FN condition (left bars). However for the group assigned to the nicotine S^Δ/FP condition (right bars), magazine entry rates during the CS under the nicotine FP were significantly greater than the saline FN [t(7) = 2.77; p = .028].

3.2.2.3. Inter-trial-interval magazine entry rates. The ITI magazine entry rates are displayed in Fig. 3 bottom. A 2 (groups) X 2 (drug conditions) repeated measures ANOVA revealed a marginal effect for group [F(1,14) = 3.85; p = .070; η^2 = .22] a significant effect for drug conditions [F(1,14) = 4.868; p = .045; η^2 = 0.26] and a significant group X drug condition interaction [F(1,14) = 5.531; p = .034; η^2 = 0.28]. As evident, ITI rates in the nicotine FN condition exceeded the saline FP condition for the nicotine FN trained rats (left bars), and also differed from the ITI rates in the nicotine FP and saline FN conditions for the nicotine FP trained rats (right bars).

3.2.2.4. CS magazine entry rates compared to ITI magazine entry rates. Stimulus control by the CS can be determined by comparing CS response rates to the ITI response rates. For the nicotine FN/S^D group, in the nicotine FN condition the rate of responding during ITI did not significantly differ from the CS rate as revealed by the paired samples, within group *t*-test [t(7) = 1.77; p = .11]. It should be noted again here that the FN ITI rate was the highest of all conditions, as noted above, and this lack of difference reflects elevation rather than suppression of food magazine entry during the CS and ITI under nicotine that concurrently functioned as the operant S^D. Conversely, for this same group, the rate of responding under saline (FP/S^Δ) to the CS was significantly greater than during the ITI [t(7) = 6.906; p < .001]. Within group, paired samples *t*-tests were conducted. For the group trained with the nicotine FP/S^Δ (right bars) magazine entry rates during the CS were significantly greater than during the ITI under the nicotine FP [t(7) = 7.96; p < .001] and under the saline FN [t(7) = 3.18; p = .015].

4. Discussion

The present investigation sought to determine: 1) if the interoceptive stimulus effects of nicotine, with saline counterbalance, can simultaneously function within subjects as an operant S^D for lever-press→ food reinforcer and as a Pavlovian FN for CS-light→ no-food US relations; and 2) if nicotine can simultaneously function as an operant S^Δ for response→ no reinforcer and as a Pavlovian FP for CS→US relation. There was clear evidence for operant S^D/S^Δ stimulus control, Pavlovian FP stimulus control with nicotine and saline, but mixed evidence for FN stimulus control.

Nicotine sustained stimulus control as an S^D and as an S^Δ as indicated during training sessions, but most critically by the results of the two sets of non-reinforcement tests, one set conducted after 16 sessions of training and the other conducted following the 34th training session. These results are consistent with all of our prior work, and further validate the utility of the go/no-go one-manipulanda drug discrimination procedure for evaluating interoceptive associative stimulus control phenomena with the discriminative stimulus effects of nicotine (see Troisi, 2013a, 2015). It appears that concurrently imposing opposing Pavlovian feature occasion-setting contingencies on operant response-reinforcer contingencies does not undermine operant stimulus control by nicotine. More specifically, simultaneously arranging nicotine to function as a FN state for the CS-light→ no-food relation did not appear to undermine nicotine's S^D function for lever-pressing→ food relationship. Conversely, simultaneously arranging nicotine to function as a Pavlovian FP for CS-light→ food relation did not undermine nicotine's S^Δ function in suppressing lever pressing. Nicotine functioned effectively as S^D and S^Δ.

The Pavlovian feature results for goal tracking were mixed. Critically, the 8 s light increased rates of magazine entry relative to the ITI in all conditions except the nicotine FN condition, suggesting that the light effectively functioned as a CS in evoking conditioned magazine entry. Nicotine appeared to function relatively well as an FP - but not an FN. Saline also functioned effectively as an FP but somewhat less effectively as a FN. First, for the nicotine S^Δ/FP trained rats, although saline FN magazine entry during the CS was significantly lower than during the nicotine FP, such saline FN responding during the CS was also greater than during the ITI. Second, there was no difference in magazine entry during the CS in the nicotine FN and saline FP conditions for the nicotine S^D/FN trained rats, and ITI magazine entry rates were elevated with the nicotine FN. These results suggest the lack of inhibitory control with the nicotine FN condition and weak inhibitory control with the saline FN condition. Third, by comparison, with the nicotine FP for the nicotine S^Δ/FP trained rats, the rate of magazine entry during the CS was significantly greater than the rate during the ITI; this was also the case with the saline FP for the nicotine S^D/FN trained rats. The nicotine S^D appeared to accentuate magazine entry for the nicotine S^D/FN group, and the rate of responding during the target CS was also elevated with the nicotine FN. Nicotine's FN effect appeared to be undermined by its concomitant S^D effect by raising overall magazine entry rates during the ITI and the CS - perhaps following periods of lever pressing. Therefore, the possibility that during S^D/FN training, coincidental presentation of food pellets (which were the result of lever pressing during the CS) attenuated (but did not eliminate) the negative contingency between the CS light and the food-US cannot be ruled out. A future investigation might arrange to postpone operant reinforcement during the CS-. By contrast, nicotine and saline S^Δ effects did not undermine their FP effects.

That Pavlovian FP control by nicotine or saline did not undermine concomitant S^Δ effects on suppressing lever pressing is interesting to note here because during the operant S^Δ sessions food pellets were dispensed only following the 8 s CS-light presentations. Under other circumstances, non-contingent food delivery (i.e., those not preceded by a discrete CS) during operant S^Δ training would be expected to attenuate the development of response suppression by perpetually reinstating responding. However, inspection of the training data (Fig. 1) shows typical drug discrimination acquisition found in our prior work. Thus, as apparent in the present study, superimposing CS→US contingencies within operant S^Δ training has little or no effect on operant S^D/S^Δ discrimination (c.f., Hammond and Weinberg, 1984). The processes appear to be orthogonal. On balance, during FN training, lever pressing produced the primary reinforcer on the same variable interval, which likely reinstated magazine entry during the ITI and CS. Thus, although the Pavlovian and operant contingencies may co-occur independently, their effects are interactive.

It has been proposed elsewhere that operant S^D and Pavlovian FP stimuli may share a common hierarchical associative function (Davidson et al., 1988); indeed, their transfer data noted above support this view. That work however did not evaluate the extent to which the operant S^Δ and Pavlovian FN stimuli transfer across paradigms; and, to our knowledge, there is no report in the literature. Interestingly, with pigeons' autoshaped keypecks to target CS keylights, Rescorla (1987) demonstrated that diffuse noise or tone Pavlovian facilitators (feature positive) undermines the development of conditioned inhibition (feature negative) over responding to target keylight CSs. Carrying out

initial CI training retards subsequent facilitation training. Moreover, if a diffuse CI (e.g., white noise) is combined with a diffuse facilitator (e.g., a tone), there is neutral responding to a transfer target CS keylight (i.e., modulatory cancelation). These results strongly supported the view that FP and FN are modulatory opponents that are distinct from excitation promoted by target CSs. In the current study, FP vs. S^{Δ} were pitted against each other, as was FN vs. S^{D}, within session. We are currently planning an additional study that alternates Pavlovian and operant sessions to determine if FN effects develop more readily in comparison to a group that only undergoes Pavlovian or operant training. Another possibility is to carryout Pavlovian or operant training first and then examine the development of stimulus control in the opposing learning paradigms. Clearly, the current investigation represents a first step toward more clearly delineating Pavlovian–operant stimulus control interaction with interoceptive drug states.

If operant S^{D}/S^{Δ} and Pavlovian FP/FN share common hierarchical stimulus control functions, one might not have predicted the FP effects shown in present study because, presumably, the concurrent S^{Δ} training theoretically should have cancelled the FP training, and perhaps vice versa. However, in Rescorla's (1987) studies: A) one feature was established as a facilitator and the other feature was established as conditioned inhibitor; B) the CI and facilitators were brief 15 s diffuse noise or tone stimuli that preceded a 5 s target keylight; and C) none of those studies arranged for one stimulus to be both a CI and a facilitator. In the present study, the drug states functioned more like interoceptive contexts in which operant and Pavlovian conditioning were co-occurring, albeit in opposition.

Perhaps the present study possesses some aspects of an ambiguous discrimination procedure in which the feature is both a feature positive and negative as a function of the target stimulus. Using an operant procedure with rats, Holland (1991) arranged for one stimulus "X" to be followed by another stimulus "A", and on such trials lever pressing was reinforced following "A"; on other trials "A" alone was not preceded by "X" (X→A+/A-), but lever pressing was not reinforced following it (feature positive training). On feature negative training, "X" was followed by "B" but lever pressing was non-reinforced, whereas when "B" was presented alone lever pressing was reinforced (X→ B−/B+). He found that the feature functioned as FP or FN to two differing transfer targets that were in other ambiguous discrimination procedures. Holland suggested that the feature's modulatory status was conditional on which target was present (i.e., a conditional discrimination). In the present experiment, S^{D}/FN and S^{Δ}/FP stimulus roles were conditional on the drug/no-drug interoceptive contexts.

Regarding context control (e.g., Troisi, 2003b, 2011): Bouton et al. (1990) demonstrated that the benzodiazepine chlordiazepoxide (i.e., Librium) functioned effectively as a context in which extinction of fear was dependent. Interestingly, when extinction was carried out in the drug context, a return to the non-drug context *renewed* fear – a phenomenon referred to as ABA renewal. In the present investigation, it is plausible to assume that the drug and non-drug states functioned concomitantly as Pavlovian and operant contexts in which responding to exteroceptive stimuli underwent reinforcement and extinction. Indeed, Bouton et al. (2014) suggested that contextual control over discriminated operant responding might differ from Pavlovian conditioning; however, this was in reference to exteroceptive context control. The extent to which this idea operated in the present investigation with interoceptive contexts is unknown. Clearly alternating across contexts during reinforcement and extinction differs from carrying out acquisition in one context and then extinction in another, such training would not favor context discrimination, but perhaps state-dependent learning. Future investigations should opt to carry out both FP and S^{D} training in one drug state and FN/S^{Δ} in another to determine extinction processes.

One important caveat in the present investigation is that the rats were pre-exposed to nicotine in a pilot study involving nose-poking and circadian shifts. There was no theoretical rationale for the inclusion of non-naïve rats in the present study, but rather, the laboratory made use of the limited resources that were available at the time. Importantly, nicotine and non-drug states were never reliably predictive of food reward for nose-poke responses; in fact, stimulus control was not attained, which likely rendered nicotine and saline associatively neutral for the present investigation. Furthermore, both the nicotine and non-nicotine states were presented equally often in that prior work, allowing the possibility of equal opportunity for latent inhibition to emerge under the nicotine and saline conditions. Nonetheless, for this reason, a novel operant (i.e., lever pressing) was established that had never previously undergone drug discrimination training. Troisi et al (2010) have reported that the drug→response relationship is most critical for operant drug discrimination training rather than the drug→reinforcer relationship; hence the use of lever pressing rather than nose-poking in the present study; a drug→lever press relationship had never been previously established. Furthermore, these rats were never exposed to the CS light prior to the present investigation, so the drug→light relationship was novel. Regarding latent inhibition: it is possible that pre-exposure to nicotine retarded the development of FN discrimination learning (e.g., Rescorla, 1971), but it is interesting that there was no clear deficit in the FP discrimination. Even more interesting, was the finding that the operant discrimination appeared unaffected by the rats' prior exposure to nicotine; and it is unlikely that such pre-exposure augmented discrimination learning because the present data are consistent with previous work from this lab (i.e., 18 sessions is sufficient for drug discrimination development). A study that systematically manipulates pre-exposure to nicotine on subsequent FP/FN and S^{D}/S^{Δ} discrimination learning is warranted in view of the present study's methodology.

Finally, interoceptive states interact with exteroceptive cues in modulating behavior as shown in the present investigation. From a more translational perspective, drug seeking and drug taking behavior represent a complex behavioral repertoire with both antecedent stimulus conditions (interoceptive and exteroceptive) and consequential outcomes (Troisi, 2013b,c). Although they can function independently, Pavlovian and operant contingencies ultimately interact in modulating drug seeking and drug self-administration (Troisi, 2013b,c; Hogarth and Troisi, 2015). Here we showed how interoceptive stimulus control by nicotine simultaneously functions in dual antecedent roles in co-modulating operant and Pavlovian-evoked goal-directed behavior.

Conflict of interest

The authors report no conflict of interest.

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