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Pavlovian Discriminative Stimulus Effects of Methamphetamine in Male Japanese quail (*Coturnix japonica*)

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Abstract

Pavlovian drug discrimination (DD) procedures demonstrate that interoceptive drug stimuli may come to control behavior by informing the status of conditional relationships between stimuli and outcomes. This technique may provide insight into processes that contribute to drug-seeking, relapse, and other maladaptive behaviors associated with drug abuse. The purpose of the current research was to establish a model of Pavlovian DD in male Japanese quail. A Pavlovian conditioning procedure was used such that 3.0 mg/kg methamphetamine served as a feature positive stimulus for brief periods of visual access to a female quail and approach behavior was measured. After acquisition training, generalization tests were conducted with cocaine, nicotine, and haloperidol under extinction conditions. SCH 23390 was used to investigate the involvement of the dopamine D₁ receptor subtype in the methamphetamine discriminative stimulus. Results showed that cocaine fully substituted for methamphetamine but nicotine only partially substituted for methamphetamine in quail. Haloperidol dose-dependently decreased approach behavior. Pretreatment with SCH 23390 modestly attenuated the methamphetamine discrimination suggesting that the D₁ receptor subtype may be involved in the discriminative stimulus effects of methamphetamine. The findings are discussed in relation to drug abuse and associated negative health consequences.

Keywords

Methamphetamine; Feature Positive; Drug Discrimination; Discriminative Stimulus; Sexual Motivation; Approach Behavior; Japanese quail

Drug discrimination (DD) techniques rely on the ability of drugs to function as discriminative stimuli via their central and/or peripheral pharmacological actions (Colpaert, Niemegeers, & Janssen, 1976). These pharmacological actions are thought to produce a set of interoceptive stimuli or cues that may be used to disambiguate a set of differential reinforcement contingencies (Colpaert et al., 1976). From a drug abuse perspective, DD techniques are an effective means to experimentally evaluate abuse liability and screen candidate pharmacotherapies for drug dependence in both humans and nonhuman animal

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subjects (Carter and Griffiths, 2009; Stolerman, Childs, Ford, & Grant, 2011). Tests of stimulus substitution in subjects trained to discriminate a drug with known abuse potential allow inferences to be made about the abuse potential of novel drugs (Solinas et al., 2006). Furthermore, the underlying receptor pharmacology of a particular drug may be determined *in vivo* with a high level of pharmacological specificity with DD procedures and may serve to identify potential therapeutic targets (Solinas et al., 2006).

Pavlovian DD has emerged as a functionally and theoretically distinct method to investigate the stimulus control of behavior by drug states (e.g., Palmatier, Wilkinson, Metschke, & Bevins, 2005; Troisi & Akins, 2004; Wilkinson, Li, & Bevins, 2009). Previous research has used Pavlovian DD to investigate the discriminative stimulus effects of methamphetamine in rats. Reichel, Wilkinson, and Bevins (2007) trained one group of rats with methamphetamine (0.5 mg/kg intraperitoneally [ip]) as a feature positive (FP) stimulus signaling 4-s access to sucrose solution (unconditional stimulus; US) after presentation of a target conditional stimulus (CS). During saline sessions, no access to sucrose was provided. In a separate group of rats, methamphetamine (0.5 mg/kg ip) was trained as a feature negative (FN) stimulus that signaled sucrose solution would be withheld after the target CS whereas saline treatment signaled the availability of sucrose following presentation of the target CS. Across discrimination training, rats in the FP group showed greater head-entries into the sucrose dipper during presentation of the target CS following treatment with methamphetamine relative to saline. In contrast, rats in the FN group made fewer head-entries during CS presentation following an injection of methamphetamine compared to saline (Reichel et al., 2007). The resultant differential patterns of conditioned approach behavior suggest that stimulus control over responding is modulated by the presence or absence of the interoceptive effects of the drug (e.g., Palmatier et al., 2005; Reichel, Wilkinson, & Bevins, 2007; Troisi & Akins, 2004).

Reichel and colleagues (2007) also conducted generalization tests to determine whether drugs with a similar neuropharmacological profile would produce a similar internal state to that of methamphetamine. They found that cocaine and bupropion increased responding in the FP group and decreased responding in the FN group. In contrast, the μ -opioid receptor antagonist naloxone did not elicit methamphetamine-like responding in rats in either group. Collectively, these findings suggest that drugs that enhance monoaminergic neurotransmission may produce a similar internal state to that of methamphetamine (i.e., stimulus substitution or generalization).

Although rodents and pigeons are typically used in preclinical DD studies, Japanese quail are an interesting alternative for several reasons. Japanese quail have a well-adapted visual system and are capable of color-vision and high visual acuity (see Mills, Crawford, Domjan, & Faure, 1997 for review). In humans, visual cues are thought to become associated with drug use via aberrant learning, which is considered a key component of the addiction process (Culbertson et al., 2010; Robinson & Berridge, 2003; Tolliver et al., 2010). Male quail also have been used extensively in studies of Pavlovian conditioned behavior (e.g., Akins, Domjan, & Gutierrez, 1994; Domjan, Lyons, North, & Bruell, 1986; Hilliard, Nguyen, & Domjan, 1997). Finally, quail have been used in studies that investigate behaviors relevant to drug abuse and addiction such as locomotor sensitization, conditioned

place preference, and the effects of psychostimulants on sexual motivation and performance (Akins & Geary, 2008; Bolin & Akins, 2009, 2012; Bolin, Cornett, Barnes, Gill, & Akins, 2012; Geary & Akins, 2007; Levens & Akins, 2004; Rosine, Bolin, & Akins, 2009).

Troisi and Akins (2004) provided evidence for DD in male Japanese quail using cocaine as a serial FP and FN in a Pavlovian conditioned approach procedure. In this study, one group of male quail received repeated pairings of a red block (target CS) with copulatory reinforcement (US) following treatment with cocaine (FP group). On alternate sessions, treatment with saline was followed by presentation of the target CS and no copulatory reinforcement. A separate group of male quail (FN group) was treated similarly except the drug treatment conditions were reversed (i.e., saline was paired with copulation and cocaine was paired with no copulation). Results showed that male quail approached the target CS more under the interoceptive stimulus conditions (cocaine or saline) that were previously paired with copulation. The findings suggest that cocaine may be an effective modulator under both FP and FN conditions in a Pavlovian DD (Troisi & Akins, 2004).

The primary aim of the current study was to develop and establish a model of methamphetamine DD using a Pavlovian conditioning paradigm in male Japanese quail. Aspects of procedures from previous studies with rodents (Reichel et al., 2007) and Japanese quail (Domjan & Hall, 1986; Troisi & Akins, 2004) were used to develop the current Pavlovian DD procedure. Following DD acquisition training, tests of stimulus substitution were conducted with cocaine, nicotine, and haloperidol. The involvement of the dopamine D₁ receptor subtype was investigated with the specific D₁ receptor antagonist SCH 23390 before treatment with the 3.0 mg/kg training dose of methamphetamine. Visual access to a female quail served as the US because male quail show robust approach behavior to a narrow window through which they can see a female (Domjan & Hall, 1986).

Methods

Subjects

Four (N = 4) adult male Japanese quail served as subjects in the current experiment. Initially, a total of twenty (N = 20) adult male quail were selected as potential subjects but sixteen of these quail did not meet criteria during initial training and screening and were excluded from the study. Ten quail failed to meet the criterion during the US training phase. Of the ten quail remaining, only 4 met criteria to begin DD acquisition training. One bird (quail 171) was removed from the study during the DD testing phase due to illness. Testing was discontinued in one subject (quail 169) following testing with haloperidol because discriminated approach behavior was not maintained.

Quail (hatched from eggs obtained from Northwest Gamebirds, Kennewick, WA) were raised in brooders in mixed-sex groups until approximately 28 days. They were then individually housed in wire-mesh cages and were maintained on a 16-8 hour light-dark cycle in a temperature- and humidity-controlled vivarium at the University of Kentucky. Subjects were initially selected on the basis of the results of a single 5 min copulatory pretest to ensure they were sufficiently sexually motivated. Previous research has shown that male quail that do not copulate after 5 min are not likely to do so (Schein, Diamond, & Carter,

1972). A separate group of adult females ($N = 10$) served as visual stimuli and copulatory partners. Access to food and water was made available ad libitum.

Apparatus

An apparatus described previously by Domjan and Hall (1986) was modified slightly for use in the current study and is diagrammed in Figure 1. The apparatus had white wooden walls and the floor was covered with corrugated white paper. It consisted of a main experimental chamber (60.96 cm long $\times$ 60.96 cm wide $\times$ 30.48 cm deep) and a smaller side chamber (30.48 cm long $\times$ 30.48 cm wide $\times$ 30.48 cm deep) that was located on one side of the main chamber and was used to house a female. Both chambers were covered with gray fiberglass screening to prevent quail from jumping out of the apparatus and to allow video collection from overhead. A narrow horizontal window (15.24 cm wide $\times$ 1.27 cm tall) was located on the common wall to provide periodic visual access to the side chamber and was approximately 15.24 cm above the floor. The window was covered by a hinged piece of wood that served as a blind to restrict visual access to the side chamber unless dictated by the experimental procedure. The female was placed in a small clear Plexiglas chamber while in the side chamber to prevent her from moving out of view and/or interacting with the male through the narrow window. A blue light emitting diode (LED) served as the target CS and was located approximately 15.24 cm above the floor to the left of the narrow window. A separate self-contained Arduino UNO computer board (Arduino SA, www.arduino.cc) was used to run the program that controlled the illumination of the target CS and a small servo motor that opened and closed the window blind. An area that comprised approximately 33% ($1/3^{\text{rd}}$) of the main chamber nearest the wall where the stimulus light and window were located was defined as the goal zone and was used to quantify approach behavior (see Figure 1). Data were collected with ANY-maze Video Tracking System version 4.82 (Stoelting Co., Wood Dale, IL, USA) and from video recordings.

Drugs

d-Methamphetamine hydrochloride (provided by the National Institute on Drug Abuse, Bethesda, MD), (-)-cocaine hydrochloride, (-)-1-Methyl-2-(3-pyridyl)pyrrolidine (+)-bitartrate salt (nicotine), 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one (haloperidol) and R(+)-SCH 23390 hydrochloride were used in the current study. USP grade haloperidol injectable solution (5 mg/ml) was obtained through the University of Kentucky Department of Laboratory and Animal Resources (DLAR) and was diluted with 0.9% NaCl saline solution to achieve the appropriate dose. All other drugs were dissolved in 0.9% NaCl saline. All drugs were injected intraperitoneally (ip) at a volume of 1 ml/kg. The pH of nicotine was adjusted to 7.4.

Pavlovian DD Training Procedures

Initial training and screening—Initial training and screening consisted of two phases. During the first phase (i.e., US training), males were placed in the main experimental chamber and a female was placed in the side chamber. The window blind was manually opened and remained open to allow males visual access to the female (US) for the entire 20 min US training session and time spent in the goal zone was recorded. Unconditional

stimulus training sessions were conducted for 3-5 days and only males that spent >50% (10 min) of the session looking through the window (i.e., peeping behavior; see Dependent Measures below) moved to the next screening phase.

The second phase (i.e., CS-US training) was used to introduce the binary relationship between the stimulus light (target CS) and the window opening (and subsequent visual access to the female; US). During this phase of pretraining, the male was placed into the main experimental chamber and a female was placed into the side chamber. Each trial was followed by an intertrial interval determined under a variable-time 120-s (VT 120-s) schedule of reinforcement. During each trial, the target CS was presented for 15-s. After 15-s, the target CS was extinguished and the window blind was immediately opened for 15-s to allow the male to view the female (US). After 15-s, the window blind was closed until the occurrence of the next trial as determined by the VT 120-s schedule. Males that learned to approach the window following presentation of the stimulus light and spent the majority of the time looking at the female while the window was open (>50%) were selected to move on to the methamphetamine DD acquisition training phase. This phase of pretraining was conducted once daily for 2-3 days and sessions lasted 20 min. Approach and peeping behavior were verified from video prior to selecting males to begin DD acquisition training. The training criteria in both phases of pretraining were chosen because males that spent >50% of the total time the window was open viewing the female (i.e., peeping) represent behavior above chance levels.

Drug discrimination acquisition training—Drug discrimination acquisition training sessions were procedurally similar to the second phase of pretraining (i.e., CSUS training). Males were injected intraperitoneally (ip) with the training dose of 3 mg/kg methamphetamine or saline 15 min before each session. A similar pretreatment time has been used in previous methamphetamine DD studies with rats (Munzar & Goldberg, 2000; Reichel et al., 2007). On days that males received an injection of methamphetamine, a female was housed in the side chamber. During saline sessions the side chamber was empty and a decoy female was placed out of view in a separate plastic chamber that was covered by a sheet to control for auditory or olfactory stimuli that may be produced by the female. Therefore, the interoceptive stimuli produced by 3.0 mg/kg methamphetamine served as a feature positive stimulus that predicted visual access to a female.

It is important to note that the stimulus light (target CS) was not a redundant cue that predicted visual access to a female during DD training. Visual access to a female in the side cage was only predicted by the presence of the methamphetamine interoceptive stimulus. Treatment order was determined semi-randomly except that subjects could receive the same treatment for no more than three consecutive sessions (i.e., three consecutive methamphetamine sessions were followed by a saline session and vice versa). Sessions lasted 20 min and consisted of approximately 4-12 trials under the VT 120-s schedule. Drug discrimination acquisition training sessions were conducted once daily, 5 days per week.

Discrimination criteria—Dependent measures were approach during the CS period when the stimulus light (i.e., target CS) was illuminated and time spent peeping (see Dependent Measures section below). Discrimination criteria were operationally defined as spending

greater than 40% of the time looking at the female (i.e., peeping) while the window was open (i.e., US period) during methamphetamine training sessions and a ratio of 2:1 time spent looking on drug versus saline days across 4-5 training sessions (i.e., one week). These criteria were selected on the basis of pilot work that indicated that quail that met these criteria were indeed altering their peeping behavior based on the presence or absence of the drug stimulus.

Dependent Measures

CS period—Time spent in the goal zone during the target CS period was scored from video as the primary dependent measure during acquisition and testing. The male was considered to be in the goal zone when at least 50% of its body was located within the borders of the goal zone. The percentage of approach to the goal zone during the CS period was calculated by dividing the time spent in the goal zone while the target stimulus was illuminated across the session by the total time the stimulus light was on (i.e., $[\text{time in goal zone during CS} \div \text{total CS time}] \times 100$). A percentage score was used because the number of trials varied between sessions.

US period—Time spent looking through the narrow window into the side chamber during the US period (i.e., when the window was open) was also scored as a secondary dependent measure. This secondary measure was used to ensure males engaged the US and as an index of motivation to view a female. This “peeping behavior” was operationally defined as the head of the male being within approximately one body length of the narrow window and oriented in such a way that the beak was within a span of approximately 190-200° facing toward the window. This definition was used because quail have eyes spaced wide on the head and the best visual acuity is achieved by turning the head to the side and using monocular vision (see Mills et al., 1997). The percent time peeping was calculated similarly to the percent time in the goal zone described above.

Pavlovian DD Testing Procedures

Once a subject met criterion performance, the test phase began. Test sessions were similar to DD acquisition training sessions except the opening of the narrow window was covered with opaque translucent tape. This prevented the males from being able to see into the side chamber. All quail were therefore tested under extinction conditions because no female was present in the experiment room during any test session. Initially, test sessions were conducted weekly (on Fridays) and DD maintenance training sessions were conducted Monday through Thursday. During initial maintenance training sessions, subjects received 2 saline and 2 methamphetamine maintenance sessions in randomized order. Similar procedures have been used in previous DD studies (e.g., Munzar & Goldberg, 2000). Later, quail could potentially be tested twice per week during an accelerated testing phase. During the accelerated testing phase, quail were given two opportunities each week to test (Wednesdays and Saturdays) if they met discrimination criteria during the preceding drug and saline maintenance session (one of each) prior to the potential test day. If a subject did not meet testing criteria (same as DD criteria defined above), a remedial maintenance training session was conducted instead of the test session. The treatment administered during the remedial session was based on individual performance during the preceding maintenance

sessions. For example, if a subject failed to meet the 40% criterion on methamphetamine training days, that subject received a methamphetamine remedial session. Similarly, if the subject met the 40% peeping criterion on methamphetamine training days but did not meet the 2:1 ratio requirement on methamphetamine vs. saline sessions, then a remedial saline session took place. If a subject failed to meet criteria under both treatment conditions, no test was conducted and maintenance training resumed the following week. Subjects that failed to meet testing criteria for more than three consecutive weeks were given one week of rest where they remained in their home cage. After the rest period, quail resumed maintenance training for two additional weeks. If criteria were not met during this two-week period of training, sessions were discontinued because the discrimination was not maintained.

Generalization test procedures—A series of generalization tests were conducted with a range of doses of cocaine, nicotine, and haloperidol. These drugs were chosen for generalization testing because previous DD work has shown that cocaine and nicotine share discriminative stimulus effects with methamphetamine in rats and aves (e.g., Desai & Bergman, 2010; Gatch, Flores, & Forster, 2008; Sasaki, Tatham, & Barrett, 1995). Haloperidol was chosen as a negative control because of its antagonist effects at dopamine receptors and haloperidol has been shown previously to attenuate the discriminative stimulus effects of amphetamine and methamphetamine (Callahan, Appel, & Cunningham, 1991; Gatch et al., 2008). Full substitution was defined as >60% approach to the goal zone, partial substitution was defined as 20-60% approach to the goal zone, and drugs that elicited <20% approach to the goal zone were considered not to substitute for the methamphetamine stimulus. These criteria were chosen on the basis of magnitude of approach behavior observed during acquisition and maintenance training sessions.

Cocaine: Several doses of cocaine were selected on the basis of previous research with quail (e.g., Troisi & Akins 2004). The doses tested were 1.0, 3.0, 5.6, and 10.0 mg/kg ip and the test order for each dose was determined according to a Latin square design. On the test day, quail received an injection of cocaine and were placed back into their home cage for 10 min. After 10 min, they were tested as described above. A 10 min pretreatment interval was chosen on the basis of previous behavioral work with cocaine in quail that suggested the peak behavioral effects of cocaine generally occurs within the first 30 min following administration (Geary & Akins, 2007).

Nicotine: A similar procedure was used to test a range of doses of nicotine. The doses of nicotine used were 0.1, 0.3, 0.56, and 1.0 mg/kg ip and were selected based on a previous conditioned place preference study with quail (i.e., Bolin et al., 2012). The testing order of each dose of nicotine was determined according to a pseudo-Latin square design. On nicotine test days, quail received an injection of the appropriate nicotine dose 15 min prior to the outset of the test session and were then tested as described above.

Haloperidol: Haloperidol was tested for its ability to substitute for the discriminative stimulus effects of methamphetamine as a negative control. Two doses (0.1 and 0.5 mg/kg ip) were chosen based on previous drug discrimination research with rodents (Gatch et al.,

2008) and were administered to quail across 2 test sessions in randomized order. A 15 min pretreatment interval preceded each test.

D₁ receptor pharmacology test procedures—The specific dopamine D₁ receptor antagonist SCH 23390 was selected to determine the involvement of the D₁ receptor subtype in the discriminative stimulus effects of the 3.0 mg/kg training-dose of methamphetamine. The doses of SCH 23390 tested were 0.01 and 0.056 mg/kg ip and were selected based on previous methamphetamine drug discrimination research with rats (Munzar & Goldberg, 2000). Briefly, quail received an injection of SCH 23390 25 min prior to the start of the session followed by an injection of 3.0 mg/kg methamphetamine 15 min prior to the start of the test session. After this 25 min period, quail were tested as described above. The testing order for each dose of SCH 23390 was randomly determined for each subject.

Results

Acquisition

Percent approach to goal zone—Figure 2 (panels A-D) illustrates individual acquisition data expressed as percent time spent in the goal zone during the CS period. The data presented are from the first and last 10 methamphetamine and saline sessions (i.e., early vs. late trials) during the acquisition phase of the current experiment. Overall, two quail appeared to acquire the discrimination relatively well but there were individual differences in the rate or degree of acquisition and length of time the discrimination was maintained. Specifically, quail 608 and 605 (panels A and B) appeared to spend more time in the goal zone following treatment with 3.0 mg/kg methamphetamine relative to saline during the last 6-11 sessions of the acquisition phase. In contrast, quail 169 and 171 (panels C and D) showed similar levels of approach behavior following treatment with saline relative to methamphetamine during the CS period.

Percent time peeping—Figure 3 (panels A-D) shows the percentage of time spent looking into the side chamber (i.e., peeping) during the US period (i.e., while the window was open) for each subject. The data shown in Figure 3 correspond with the same trials shown for the CS period in Figure 2. Quail 608 (panel A) showed robust peeping behavior and was highly motivated to view a female during methamphetamine training sessions relative to when no female was present in the side chamber during saline sessions. A similar pattern of responding was observed for quail 605 (panel B) except that this male tended to show somewhat less robust peeping behavior during methamphetamine training sessions. Quail 169 (panel C) appeared to also be highly motivated to view the female but spent a comparatively large percentage of the time looking into the side chamber during saline sessions when visual access to a female was not provided. By contrast, quail 171 (panel D) showed a similar magnitude of peeping behavior relative to the other subjects during early trials but this subject's peeping behavior appeared to decrease in late trials following methamphetamine administration when a female could be viewed.

Generalization Tests

Cocaine—Figure 4 (panels A-D) shows individual data from generalization tests with a range of doses of cocaine (1.0-10.0 mg/kg ip) relative to maintenance training data (i.e., baseline $\pm$ S.E.M.) for saline (S) and methamphetamine (M) during the cocaine testing period. Although there was individual variation in baseline training performance, cocaine elicited dose-related increases in approach to the goal zone in each quail. The maximum percentage of methamphetamine-like approach behavior engendered by cocaine was between approximately 60-80%. Therefore, cocaine appeared to at least partially substitute with methamphetamine in each subject and produced full methamphetamine substitution in three of four subjects following intermediate doses of cocaine (i.e., 3.0 and 5.6 mg/kg).

Nicotine—Figure 5 illustrates the percentage of approach to the goal zone during the nicotine generalization tests relative to the percentage of approach during baseline maintenance training sessions for three male quail. One subject (171) was removed from the study prior to nicotine testing due to illness. Nicotine elicited methamphetamine-like approach and appeared to partially or fully substitute for methamphetamine in two of the three quail tested. On average, quail 608 and 605 spent approximately 60-75% of the time in the goal zone when the target CS was presented following the low and high doses of nicotine. However, nicotine appeared to only partially substitute for methamphetamine in quail 169 at the low and high doses but the intermediate doses produced no substitution.

Haloperidol—Haloperidol was tested as a negative control condition and the percent approach to the goal zone for the three quail that were tested is shown in Figure 6. At the lowest dose tested (0.1 mg/kg), quail spent approximately 55-100% of the time in the goal zone when the target CS was presented. The high dose of haloperidol (0.5 mg/kg) produced minimal approach to the goal zone in two quail and modestly decreased approach by approximately 10% in the third. Therefore, at the high dose haloperidol did not substitute for the interoceptive stimuli produced by methamphetamine.

Dopamine D₁ Receptor Pharmacology Test

Figure 7 illustrates the results of the dopamine D₁ receptor pharmacology tests for the two quail that were tested. Following pretreatment with 0.01 mg/kg SCH 23390 prior to administration of the 3 mg/kg training dose of methamphetamine, quail spent approximately 45-85% of the time the stimulus light was on standing near it (see Figure 8 panels A and B). This magnitude of approach to the stimulus light was relatively similar to that observed during methamphetamine maintenance training sessions (60-85%). When pretreated with a higher dose of SCH 23390 (0.056 mg/kg ip) prior to the receiving the training dose of methamphetamine, quail showed modest decreases in approach (around 10-15%) to the goal zone relative to baseline methamphetamine responding. Therefore, it appears that treatment with the higher dose of the selective D₁ receptor antagonist SCH 23390 only modestly attenuated approach behavior elicited by the training dose of methamphetamine.

Discussion

The present findings collectively suggest that methamphetamine may function as a feature positive stimulus in a Pavlovian serial feature positive DD procedure using male Japanese quail. During acquisition training, most quail reliably spent a greater proportion of the time in the goal zone or viewing the female through the narrow window following treatment with methamphetamine. In contrast, most subjects spent less time in the goal zone or looking through the window following an injection of saline (when there was no female to view). The findings suggest that the interoceptive effects of methamphetamine predicted visual access to a potential sexual partner (US) following presentation of the target CS (i.e., the stimulus light). That the internal state produced by methamphetamine may function as a discriminative stimulus is in agreement with previous DD studies with methamphetamine (Czoty, Makriyannis, et al., 2004; Czoty, Ramanathan, Mutschler, Makriyannis, & Bergman, 2004; Munzar & Goldberg, 2000; Munzar, Baumann, Shoaib, & Goldberg, 1999; Munzar, Kutkat, Miller, & Goldberg, 2000; Reichel et al., 2007; Sasaki et al., 1995; Tidey & Bergman, 1998).

In the current experiment, relative approach to the goal zone tended to vary between individual subjects across the acquisition and maintenance phases of the current experiment. One potential explanation for the high level of individual variation in responding is that there may have been individual differences in sensitivity to (or the ability to detect) the interoceptive effects produced by 3.0 mg/kg methamphetamine. Individual variation in sensitivity to different drugs (and drug doses) is common in DD studies (e.g., Bevins, Klebaur, & Bardo, 1997) and is most evident in DD studies that present individual subject performance (e.g., Sasaki et al., 1995) rather than grouped data (e.g., Reichel et al., 2007).

In the current study, administration of cocaine elicited methamphetamine-like approach to the goal zone and appeared to fully substitute for the methamphetamine feature in each subject at the intermediate or high doses tested. These findings are in agreement with those reported in previous methamphetamine DD studies where cocaine has been shown to fully substitute for methamphetamine in rats, squirrel monkeys, and pigeons (Czoty, Makriyannis, et al., 2004; Czoty, Ramanathan, et al., 2004; Munzar et al., 1999; Tidey & Bergman, 1998; Sasaki et al., 1995). Taken together, these findings suggest that cocaine and methamphetamine may share discriminative stimulus effects.

Nicotine elicited methamphetamine-like approach behavior in the current experiment but approach behavior did not increase as an orderly function of dose. Nonetheless, nicotine did appear to fully substitute for methamphetamine in two quail and produced partial substitution in one quail. Previous DD research has shown that nicotine and methamphetamine share discriminative stimulus effects in rodents (Desai & Bergman, 2010; Gatch et al., 2008). Desai and Bergman (2010) reported that nicotine dose-dependently substituted for the discriminative stimulus effects of methamphetamine with the 0.1 mg/kg dose of nicotine engendering full methamphetamine substitution. One potential explanation for the disorderly pattern of substitution by nicotine in the current study may be that acute nicotine administration produced locomotor hypoactivity. Comparatively lower levels of general locomotor activity were observed during nicotine tests relative to the other

generalization tests in the current study (data not shown). Therefore, it is difficult to definitively conclude the degree to which methamphetamine generalized the nicotine stimulus in the current study.

The nonspecific dopamine receptor antagonist haloperidol was tested as a negative control in the present study and methamphetamine-like approach behavior was observed at the low dose but not the high dose. There are no published studies that have explicitly tested whether methamphetamine generalizes the haloperidol interoceptive stimulus. In one study, haloperidol (0.05 or 0.5 mg/kg ip) fully antagonized the discriminative stimulus effects of methamphetamine (Gatch et al., 2008), but haloperidol was not tested alone. General locomotor activity was decreased by haloperidol in the present study (data not shown), thus it is difficult to determine if changes in approach to the goal zone reflected motor disturbances as opposed to changes in discriminated approach behavior. Nonetheless, the present findings corroborate those of previous studies to suggest that the dopamine receptor system is likely involved in the discriminative stimulus effects of methamphetamine.

Pretreatment with the selective dopamine D₁ receptor antagonist SCH 23390 (0.056 mg/kg) only modestly attenuated the discriminative stimulus effects of the 3.0 mg/kg training dose of methamphetamine in the current study. These findings are relatively consistent with previous findings from methamphetamine DD studies in rodents. Munzar and Goldberg (2000) reported that pretreatment with a 0.056 mg/kg dose of SCH 23390 fully attenuated the discriminative stimulus effects of methamphetamine (1 mg/kg) in rats trained to discriminate methamphetamine versus saline using an operant DD procedure. One explanation of the relatively small decrease in methamphetamine-like responding in the current study may be that SCH 23390 produced motor impairments. Pretreatment with SCH 23390 appeared to dose-dependently decrease locomotor responses to 3.0 mg/kg methamphetamine in the current study (data not shown). Alternatively, a higher dose of methamphetamine (3.0 mg/kg) was used in the present study compared to in previous studies. Attenuation of the interoceptive effects of methamphetamine by SCH 23390 is likely to be dose-dependent (Munzar & Goldberg, 2000). However, inherent methodological differences make it difficult to reconcile the somewhat contradictory findings between these studies. The current findings should be considered preliminary but appear to suggest the involvement of dopamine D₁ receptors in the discriminative stimulus effects of methamphetamine in quail using a Pavlovian DD procedure.

Other species differences between aves and mammals may have also contributed to the somewhat discordant findings between previous studies with rodents and the current work with Japanese quail. First, previous comparative research with rodents and birds indicates that birds have a higher ratio of dopamine D₂ receptors relative to the D₁ receptor subtype compared to rodents (Richfield, Young, & Penney, 1987). It is possible that the enhanced ratio of D₂ receptors in birds may have altered the interoceptive effects produced by methamphetamine. This would have potentially altered the nature of the methamphetamine discriminative stimulus and subsequently altered DD performance in the current study. Although this explanation is plausible, there is currently no research with Japanese quail that has examined the neuropharmacological effects of drugs on neurotransmitter release (e.g., with *in vivo* voltammetry or microdialysis). Additional research is needed to determine

whether there are species differences in neurophysiological responses to methamphetamine between aves and mammals.

Secondly, there may be species differences in the rate at which methamphetamine or other drugs are metabolically degraded and cleared from the body between rats and quail. Previous research with rodents has suggested that the elimination half-life of methamphetamine in rats is approximately 1 hour (Rivière, Byrnes, Gentry, & Owens, 1999). Since metabolism and clearance data for methamphetamine are not available in male Japanese quail, it is difficult to determine whether differences in the rate of metabolism or clearance of methamphetamine in quail may have affected their performance in a DD task. However, behavioral studies with quail suggest that the rate of onset and duration of the behavioral effects of psychostimulants are relatively similar in quail and rodents (e.g., Geary & Akins, 2007).

The present work expands the existing Pavlovian DD literature (e.g., Reichel et al., 2007) and work with Japanese quail (Troisi and Akins, 2004) to show that a nonconsummatory US may maintain behavior in Pavlovian DD studies. Food has served as the primary reinforcer or US in the majority of preclinical DD studies to date. Copulation served as the primary US in the previous Pavlovian DD study with quail (Troisi & Akins, 2004). The current experiment utilized visual access to a female quail. The primary advantage of a non-consummatory sexually-relevant US is that multiple trials may be conducted in succession within a single session without intervention by the experimenter. However, similar to other consummatory responses, motivation to view a female is likely to vary across individuals, time, and may also be influenced by other factors (e.g., circulating hormone levels or drug treatment). This may partly explain the increased individual variation that was observed across the study.

Despite the expansion of the extant literature on DD with quail and Pavlovian DD in general, there are several potential limitations that restrict the conclusions that may be drawn from the current study. One limitation may be that methamphetamine was only trained as a FP stimulus. Although the inclusion of a FN control condition is of theoretical interest from a learning perspective, a FN group was not included in the present study for two primary reasons. First, previous research with quail has shown that cocaine, a psychostimulant with some pharmacological similarity to methamphetamine, effectively functioned as both a positive and negative drug feature in a relatively similar Pavlovian DD procedure (Troisi & Akins, 2004). Given that cocaine and methamphetamine increase extracellular monoamines, methamphetamine could be expected to function as a FN stimulus in quail similar to cocaine. Second, the FP condition was of primary interest because this configuration more closely approximates the circumstances under which methamphetamine and high-risk sexual behavior co-occur in humans (e.g., Volkow, Wang, Fowler, Telang, Jayne, & Wong, 2007).

Another potential limitation of the present study is that a female was located in the experiment room during saline training sessions but no visual access to the female was provided. This was intended to control for the presence of auditory or olfactory cues produced by the female as these cues could also potentially serve as discriminative stimuli that signaled the presence of the female that would confound a Pavlovian DD interpretation

of the data. Although there are data to suggest that olfactory or auditory cues should not be sufficient to elicit approach behavior in male quail (Domjan & Hall, 1986), the effect of these cues on approach and/or peeping behavior during saline training sessions in the current study cannot be ruled out.

The Pavlovian DD procedure used in the present study may provide insight into the interaction between interoceptive drug stimuli and social behaviors. The current findings suggest that an internal drug state may serve as a discriminative stimulus for the availability of a sexual partner that may subsequently prime sex-seeking behavior. This may provide a potential mechanism for the increased prevalence of sexually transmitted infections among methamphetamine users (Shoptaw et al., 2013).

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Figure 1.
A diagram of the apparatus used in the present study.

Figure 2.

Individual acquisition training data expressed as the percentage of time spent in the goal zone during presentation of the target CS (i.e., CS-period) from the first and last 10 acquisition sessions (Early and Late Trials, respectively). Hashed line separates Early Trials from Late Trials.

Acquisition: US Period

Figure 3.

Individual acquisition training data expressed as the percentage of time spent looking through the narrow window (i.e., peeping behavior) during presentation of the US from the first and last 10 acquisition sessions (Early and Late Trials, respectively). Hashed line separates Early Trials from Late Trials.

Figure 4.

Individual subject data for all subjects ($N = 4$) from cocaine generalization tests expressed as the percentage of approach to the goal zone during the CS-period. Mean percent approach to the goal zone ($\pm$ S.E.M.) across baseline saline (S) and methamphetamine (M) maintenance training sessions are shown to the left (filled squares) and cocaine test data are shown to the right (open circles).

Figure 5.

Individual subject data for three subjects (N = 3) from nicotine generalization tests expressed as the percentage of approach to the goal zone during the CS-period. Mean percent approach to the goal zone ($\pm$ S.E.M.) across baseline saline (S) and methamphetamine (M) maintenance training sessions are shown to the left (filled squares) and nicotine test data are shown to the right (open circles).

Figure 6.

Individual subject data for two subjects (N = 2) from haloperidol generalization tests expressed as the percentage of approach to the goal zone during the CS-period. Mean percent approach to the goal zone ($\pm$ S.E.M.) across baseline saline (S) and methamphetamine (M) maintenance training sessions are shown to the left (filled squares) and haloperidol test data are shown to the right (open circles).

Figure 7.

Individual subject data for two subjects (N = 2) from D₁ receptor pharmacology tests with SCH 23390 tested in combination with 3.0 mg/kg methamphetamine. Mean percent approach to the goal zone ($\pm$ S.E.M.) across baseline saline (S) and methamphetamine (M) maintenance training sessions are shown to the left (filled squares) and SCH 23390 + 3.0 mg/kg methamphetamine test data are shown to the right (open circles).