Published in final edited form as:

Behav Processes. 2012 October ; 91(2): 177–183. doi:10.1016/j.beproc.2012.07.004.

Chronic pre-exposure to methamphetamine following 31 days of withdrawal impairs sexual performance but not sexual conditioning in male Japanese quail

B. Levi Bolin and Chana K. Akins*

Department of Psychology, University of Kentucky, Lexington, KY 40506-0044, USA

Abstract

In the current study, male quail were administered methamphetamine (3.0 or 5.6 mg/kg IP) or saline once-daily for 10 days and locomotor activity was assessed. Following a 31-day withdrawal period, sexual conditioning trials were conducted such that a conditioned stimulus (CS) was presented prior to a copulatory opportunity with a female quail. Male quail treated with methamphetamine (5.6 mg/kg) showed a decrease in locomotor activity from Trial 1 to Trial 10 suggesting a potential tolerance effect. Following the 31-day withdrawal period, all male quail that received the CS paired with a copulatory opportunity showed enhanced approach to the CS, regardless of treatment history. Thus, chronic pre-exposure to methamphetamine did not alter sexual conditioning. In contrast, chronic pre-exposure to methamphetamine (3.0 mg/kg) decreased the frequency of successful copulations suggesting that it impaired sexual performance. The findings suggest that methamphetamine may differentially affect the neural circuitry involved in motivational systems compared with those involved in consummatory aspects of sexual behavior. These effects may last long after drug cessation.

Keywords

Pavlovian Conditioning; Drug Tolerance; Methamphetamine; Motivation; Locomotor Activity; Sexual Behavior; Aves

1. Introduction

Individuals who use methamphetamine report heightened sexual drive (e.g., Semple et al. 2009) and human laboratory research similarly indicates that stimulant exposure may enhance sexual desire (Volkow et al. 2007). These findings have prompted the development of the working hypothesis that methamphetamine may positively alter motivational processes related to sexual behavior. However, a number of obvious ethical and practical limitations preclude the ability to test this hypothesis in humans.

Preclinical studies that have focused on the effects of methamphetamine on sexual motivation and performance are scant and those studies that exist provide conflicting

© 2012 Elsevier B.V. All rights reserved.

*Address correspondence to: Dr. Chana K. Akins, 207i Kastle Hall, Department of Psychology, University of Kentucky, Lexington, KY 40506-0044, ckakin1@uky.edu, phone: (859) 257-1103, fax: (859) 323-1979.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

conclusions. For example, female rats display enhanced sexual motivation (more proceptive and receptive displays) following 3 consecutive days of methamphetamine treatment (5 mg/kg/day; Holder et al. 2010; Holder and Mong, 2010). In contrast, male rats show a decrease in sexual motivation (i.e., increased mount and intromission latencies) and sexual performance (i.e., decreased mounts, intromissions, and ejaculations) after 3 days of methamphetamine (2 or 4 mg/kg; Frohmader et al. 2010). Similarly, male Japanese quail show a decrease in sexual motivation, as indicated by slower running time toward a female, after chronic pre-exposure (10 days) to 3 mg/kg methamphetamine (Bolin and Akins, 2009). In contrast, these same males did not show impairment of copulatory behavior. Although there are some methodological, sex, and species differences to consider, the findings of preclinical studies on methamphetamine and sexual behavior provide mixed results.

Chronic pre-exposure to drugs may result in behavioral and neurochemical changes long after drug cessation. Barr and colleagues (1999) showed that male rats exhibited decreased locomotor activity in anticipation of presentation of a female rat and increased postejaculatory intervals 12 hrs following a 4 day escalating amphetamine administration regimen. Similarly, Clemens and colleagues (2007) found that repeated intermittent exposure to methamphetamine (8 mg/kg IP) resulted in decreased social exploration behavior 49 days following cessation of methamphetamine administration. Numerous other studies have suggested that prior exposure to methamphetamine results in lasting changes in both behavior and neurochemistry measured during withdrawal (see Kitanaka et al. 2008 for review).

The Pavlovian sexual conditioning procedure has been used to investigate the effects of psychostimulants on sexual motivation in male quail. In this procedure, an arbitrary conditioned stimulus (CS) is presented and is then followed by (paired with) a copulatory opportunity with a female quail (the unconditioned stimulus or US). Following repeated pairings of the CS and US, the CS becomes a reliable predictor of a sexual encounter. As a result, male quail learn to approach and remain near the stimulus. Previous research has suggested that this conditioned approach behavior may be an index of sexual motivation that is acquired across trials (Domjan et al. 1986).

Psychostimulants have been shown to alter Pavlovian sexual conditioning. Levens and Akins (2004) reported that conditioned approach to a CS that signaled copulation with a female was enhanced in male quail previously exposed to cocaine (10 mg/kg for 6 days) relative to saline controls. In addition, sexual performance was enhanced in those males. Male quail had shorter latencies to copulate and a greater frequency of successful copulations. It is of critical importance to note that all conditioning and copulatory tests were given in a drug-free state following a 10-day withdrawal period. Therefore, enhanced conditioned approach and copulatory behavior were a relatively lasting effect of prior cocaine exposure. The findings suggest that chronic pre-exposure to stimulants may affect sexually relevant learning and sexual performance after cessation of drug administration. The goal of the current study was to extend the existing literature by investigating the effect of chronic pre-exposure to methamphetamine on sexual conditioning and to determine whether this effect was evident after an even longer withdrawal period (31 days).

2. Materials and methods

2.1. Subjects

Subjects were 45 adult male Japanese quail (*Coturnix japonica*), 2–3 months old. Eggs were supplied by Northwest Gamebirds (Kennewick, WA) and quail chicks were hatched and then raised in mixed-sex groups until approximately 28 days of age. At 28 days of age, quail were housed individually in wire mesh cages (GQF Manufacturing, Savannah, GA). Male

quail were selected for the current experiment on the basis of a pretest for sexual behavior. During the pretest, a female quail was placed in the home cage of each male for 5 min. Only those subjects that copulated were selected for the experiment as males that do not successfully copulate within 5 min are not likely to do so (Schein et al. 1972). Forty-one ($n = 41$) sexually mature female quail that were the same age as the male quail were used as copulation partners during the sexual conditioning phase. All subjects were drug and sexually naïve prior to experimentation (except for the copulatory pretest) and were maintained on long photoperiod days (16 light: 8 dark) to maintain their reproductive readiness. Food and water were available ad libitum. All experimental procedures were approved by and carried out under the guidelines set by the Institutional Animal Care and Use Committee at the University of Kentucky.

2.2. Drugs

Methamphetamine hydrochloride (METH; NIDA, Bethesda, MD) was dissolved in saline (0.9% NaCl) and injected intraperitoneally (IP) at a volume of 1 ml/kg of body mass. Doses were 3.0 mg/kg and 5.6 mg/kg. These doses were primarily chosen on the basis of previous research with METH in male Japanese quail. We chose to administer 3.0 mg/kg METH because, although they used different procedures, Bolin and Akins (2009) found impaired sexual motivation using this dose. In contrast, they found that 1.0 mg/kg did not alter sexual motivation. Therefore, a higher dose of METH (5.6 mg/kg IP) was used in the current study. This dose has been shown to enhance locomotor activity in male Japanese quail with a history of chronic pre-exposure to methylphenidate (Rosine et al. 2009).

2.3. Apparatus

2.3.1. Locomotor activity—Locomotor activity was assessed in chambers that measured 28.6 cm long $\times$ 21.2 cm wide $\times$ 21.2 cm deep (Med-Associates, St. Georgia, VT). Each chamber was equipped with six photobeams approximately 6.4 cm apart and 3.2 cm above the floor. Photobeam breaks were recorded by MedPC IV Software (Med-Associates, St. Albans, VT).

2.3.2. Pavlovian sexual conditioning—Six large Plexiglas test cages (91.4 cm wide $\times$ 61.0 cm deep $\times$ 30.5 cm tall) were used during sexual conditioning (see Figure 1). The side walls of the cages were covered with white paper and the floors of the cages were covered with white corrugated paper. A smaller Plexiglas side cage (30.5 cm wide $\times$ 61.0 cm deep $\times$ 27.9 cm tall) was attached to one end of each test cage and was used to house a female bird. A door connecting the test cage and the side cage allowed male quail to access the female's side cage. The door was covered so that males were unable to see the female unless the door was opened. A 25w red light served as the conditioned stimulus (CS). The CS light was housed inside of a white wooden box (15.24 cm wide $\times$ 13.97 cm deep $\times$ 26.67 cm tall) that had two holes (3.81 cm diameter) approximately 15.24 cm above the floor. Thus, the single light may have appeared as two lights because there were two circular holes in the box. Charcoal gray fiberglass screening was used to cover the holes to prevent male subjects from being able to see the CS light unless illuminated and prevent males from pecking the light. The wooden box was located in one corner of the test cage next to the common wall of the test cage and the female's side cage (refer to Figure 1). The wooden boxes were placed to the right of the door in half of the cages and to the left of the door in the remaining cages. A 36.6 cm $\times$ 36.6 cm area was marked on the white paper floor around each wooden box and was referred to as the CS zone.

2.4. Procedure

A diagram of the timeline of the procedures is shown in Figure 2.

2.4.1. Locomotor activity—A similar procedure was used to measure locomotor activity in the current experiment as in a previous experiment (Bolin and Akins, 2009). Male quail were injected (IP) with saline (1 ml/kg; $n = 15$), 3.0 mg/kg METH ($n = 16$), or 5.6 mg/kg METH ($n = 14$) and immediately placed in locomotor chambers. Locomotor activity was recorded for 60 min. A total of ten trials were conducted, one per day. A 10-day METH exposure period length was chosen on the basis of previous research with male Japanese quail that showed METH produced locomotor tolerance following 10 consecutive days of exposure (Bolin and Akins, 2009). White noise was used during locomotor activity sessions to attenuate extraneous noise.

2.4.2. Withdrawal period—The locomotor activity phase was followed by a 31-day withdrawal period during which quail did not receive drug and remained in their home cage. Previous behavioral research with METH has shown that chronic METH exposure decreases social behaviors in rats nearly 2 months after discontinuation of METH treatment (Clemens et al. 2007) and these behavioral changes are thought to correspond with long-term changes in neurochemistry that result from METH exposure (see Kitanaka et al. 2008 for review). However, it is currently not known whether METH has similar long-term effects on the sexual behavior system. Therefore, a 31-day withdrawal period was chosen to determine if the effects of stimulants on sexual motivation and/or copulatory behavior potentially extend beyond 10 days of drug abstinence as has been reported in previous research with Japanese quail (Levens and Akins, 2004; Bolin and Akins, 2009).

2.4.3. Pavlovian sexual conditioning—Following the withdrawal period, male subjects were randomly assigned to one of six groups: Paired-METH 5.6 mg/kg ($n = 7$), Unpaired-METH 5.6 mg/kg ($n = 7$), Paired-METH 3.0 mg/kg ($n = 8$), Unpaired-METH 3.0 mg/kg ($n = 8$), Paired-Saline ($n = 7$), and Unpaired-Saline ($n = 8$). During each conditioning trial, male quail were placed into the center of the test cage for a 30 sec pre-CS baseline period. Following the pre-CS period, the CS light was illuminated for 30 sec and was then turned off. Subsequently, for paired males, the door to the female's side cage was raised and males were allowed to enter the side cage for a copulatory opportunity with a female quail for 5 min. Unpaired males received the same treatment as paired males except they received a 5 min copulatory opportunity with a female bird in their home cage 2–3 hours prior to sexual conditioning sessions. Female copulation partners were rotated such that no male copulated with the same female twice. Fourteen sexual conditioning trials were conducted, one per day. White noise was also used to attenuate extraneous noise during sexual conditioning sessions.

It should be noted that, in sex conditioning studies that measure conditioned approach behavior, the presence of a female quail in the side cage should not increase approach due to olfactory and/or auditory cues as previous research indicates that these cues alone are not sufficient to elicit approach behavior from male quail (Domjan and Hall, 1986).

During the CS period, time spent in the CS zone was recorded whenever a male quail had two feet inside of the CS zone. During copulatory opportunities, the frequency of cloacal contact movements (CCMs) was recorded as a measure of sexual performance. Frequencies of CCMs were used as an index of sexual performance because foam and semen are transferred into the female's cloaca via the male's cloacal gland during copulation representing a successful copulatory event. CCMs are highly stereotyped behaviors that occur when the male's cloaca makes contact with the cloaca of the female.

2.5. Dependent measures and statistical analyses

Photobeam breaks were collected as the dependent measure during the locomotor activity phase of the current study. Locomotor activity data from the first and second 30 min of Trial 1 and Trial 10 were analyzed using separate 3 (Treatment) $\times$ 2 (Trials) repeated measures analyses of variance (ANOVAs). Significant effects were followed up with subsequent one-way ANOVAs and/or Fisher's least significant difference (LSD) post-hoc comparisons or paired-samples t -tests where appropriate. Data from locomotor activity trials were divided into two 30 min periods on the basis of previous research with rats that indicated peak plasma METH concentration was reached within 10 min following administration (Melega et al. 1995). Since metabolism and clearance data for METH are currently unavailable in quail, a 60 min session length was used to ensure the peak locomotor activating effects of METH were captured. During sexual conditioning, conditioned approach and CCMs were analyzed with a 3 (Treatment) $\times$ 2 (Pairing) $\times$ 14 (Trials) repeated-measures ANOVAs. Significant main effects were followed up with Fisher's LSD post-hoc tests where appropriate. Statistical analyses were conducted with IBM SPSS Statistics Software Version 20 (IBM Corporation, Armonk, New York) and statistical significance was set at the $p < 0.05$ level for all analyses.

3. Results

3.1. Locomotor activity

3.1.1. First 30 minutes—Figure 3 (upper panel) illustrates mean total photobeam breaks for Trial 1 and Trial 10 during the first 30 min of the 60 min locomotor activity sessions. Male quail that received the high dose of METH (5.6 mg/kg) demonstrated a decrease in photobeam breaks from Trial 1 to Trial 10 during the first 30 min, whereas male quail that received the low dose of METH (3.0 mg/kg) or saline did not. This resulted in a significant Treatment $\times$ Trials interaction, $F(2, 42) = 4.875$. A separate one-way ANOVA followed by a Fisher's LSD post-hoc analysis comparing treatment groups on Trial 1, revealed that male quail treated with the high dose of METH (5.6 mg/kg) had a higher frequency of photobeam breaks during the first 30 min compared to quail that received the lower dose of METH (3.0 mg/kg) or saline, $F(2, 44) = 5.790$. A separate one-way ANOVA for Trial 10 during the first 30 min failed to reveal any significant between-group differences, $F(2, 44) = 0.755$. Furthermore, male quail that received 5.6 mg/kg METH showed a significant decrease in locomotor activity from Trial 1 to Trial 10 during the first 30 min, $t(13) = 2.251$. No other effects attained statistical significance.

3.1.2. Second 30 minutes—Data from the second 30 min of trials 1 and 10 are shown in Figure 3 (lower panel). During the second 30 min, male quail treated with METH (5.6 mg/kg) had a higher frequency of photobeam breaks than quail treated with saline overall (see Figure 3, lower panel). This was evident as a significant main effect of Treatment, $F(2, 42) = 5.528$. Subsequent post-hoc analysis comparing treatment groups (collapsed across trials) indicated that quail that received 5.6 mg/kg METH had significantly higher levels of locomotor activity compared to quail that received saline but not 3.0 mg/kg METH.

3.2. Sexual conditioning

Figure 4 represents time spent in the CS zone across trials for paired (upper panel) and unpaired males (lower panel) that received pre-exposure to saline, 3.0 mg/kg or 5.6 mg/kg METH followed by a 31-day withdrawal period. Regardless of drug treatment history, male quail in the paired condition spent significantly more time in the CS zone compared to the unpaired condition. There was a significant main effect of Pairing, $F(1, 39) = 11.289$. No other main effects or interactions were statistically significant, $F_s < 1.808$.

3.3. Sexual performance

The average frequency of CCMs made by quail treated with saline, 3.0 mg/kg METH and 5.6 mg/kg METH with a female during all copulatory opportunities (collapsed across trials and pairing) is shown in Figure 5. Overall, male quail increased their frequency of CCMs (data not shown). This was evident as a significant main effect of Trials, $F(8, 330) = 4.298$. However, male quail pre-exposed to METH made fewer CCMs with a female than male quail pre-exposed to saline (regardless of pairing history), resulting in a significant main effect of Treatment [$F(2, 39) = 3.358$]. Post-hoc comparisons of treatment groups revealed that male quail treated with 3.0 mg/kg METH made fewer CCMs ($M = 2.558 \pm 0.25$) with female birds than those treated with saline ($M = 3.771 \pm 0.39$) but quail treated previously with 5.6 mg/kg METH ($M = 2.898 \pm 0.37$) were not statistically different from those treated with saline or 3.0 mg/kg METH.

4. Discussion

In the current study, administration of 5.6 mg/kg methamphetamine enhanced locomotor activity during the first 30 min of Trial 1 compared to quail treated with a lower dose of methamphetamine (3.0 mg/kg) or saline. By Trial 10, no differences in locomotor activity were apparent during the first 30 min of the 60 min session. One potential explanation for these findings may be that chronic administration of methamphetamine (5.6 mg/kg) produced locomotor tolerance in male Japanese quail. These findings may suggest that chronic methamphetamine exposure results in decreased sensitivity to the locomotor activating effects of methamphetamine (i.e., tolerance).

Bolin and Akins (2009) reported tolerance following repeated treatment with lower doses of methamphetamine (1.0 and 3.0 mg/kg IP). In the current study, a possible tolerance effect was only observed for quail that received 5.6 mg/kg while 3.0 mg/kg methamphetamine did not appear to affect locomotor activity. A possible explanation for this is that a 20 min pretreatment interval (in the home cage) was used by Bolin and Akins (2009) whereas locomotor activity was measured immediately following treatment in the current study. The differing patterns of locomotor activity observed between these studies may reflect differences in drug conditioning history. Stronger conditioning may have occurred in the home cage compared to the locomotor activity chamber in Bolin and Akins (2009). In contrast, quail in the current study were injected and immediately placed into locomotor activity chambers which may have allowed stronger conditioning to the locomotor activity chambers.

In contrast to the effects of methamphetamine on locomotor activity in quail, the majority of previous studies with rodents indicate that repeated administration of methamphetamine (IP) results in behavioral sensitization (Kitanaka et al. 2003; Milesi-Hallé et al. 2005; Shuto et al. 2006). One rodent study supports the current findings in that escalating high doses of methamphetamine and/or prolonged exposure via intravenous (IV) administration produce tolerance (Segal et al. 2005; Segal and Kuczenski, 2006). However, the procedures and route of administration between this study and the current study differ considerably. In addition, although the neurotransmitter systems appear to be highly conserved from mammals to birds, there are some reported differences. For example, birds have greater numbers of dopamine D₂ receptors relative to D₁ receptors and the reverse is true for mammals (Richfield et al. 1987). It is therefore difficult to determine whether the differing results between studies that have investigated the effects of methamphetamine on locomotor activity are due to species differences, procedural differences, and/or metabolism differences (as the pharmacokinetic profile of methamphetamine in quail is currently unavailable).

One alternative explanation for the decrease in locomotor activity from Trial 1 to Trial 10 (during the first 30 min) in the current study may be that repeated administration of methamphetamine resulted in the development of or increased occurrence of stereotyped behaviors. Previous research with rodents has shown doses of 3.0 mg/kg methamphetamine or higher may induce pronounced stereotypy (e.g., Gentry et al. 2004; Peachey et al. 1976). In contrast to rodents, it has been suggested that stereotypy is difficult to identify and measure in quail and only one study using apomorphine has reported evidence of it (Castagna et al. 1997). Furthermore, there is no evidence from previous observations in our laboratory that acute or repeated administration of methamphetamine induces stereotypy in quail (Bolin and Akins, 2009; Rosine et al. 2009). However, because locomotor activity sessions were not recorded in the current experiment, the occurrence of stereotypy cannot be ruled out in the current findings.

Interestingly, quail treated with 5.6 mg/kg methamphetamine had higher overall levels of locomotor activity compared to saline controls during the second 30 min period of testing (collapsed across trials) in the current study. Upon closer examination of the data, this difference may not necessarily reflect an increase in locomotor activity per se. As expected, quail treated with saline continued to decrease their locomotor activity across the 60 min session. In contrast, quail treated with 3.0 mg/kg and 5.6 mg/kg of methamphetamine (with the exception of the decrease in activity during Trial 1) appeared to maintain comparable levels of locomotor activity across the entire 60 min session. Therefore, it does not appear this effect represents an increase in methamphetamine-induced locomotor activity but rather that treatment with methamphetamine produced relatively constant levels of locomotion across the entire session following repeated administrations. Similar findings with regard to the time course of methamphetamine's effects on locomotor activity have been reported in previous research with mice (Ago et al. 2009).

During sexual conditioning, neither dose of methamphetamine produced differences in approach to a CS that predicted a copulatory opportunity 31 days after the final administration of methamphetamine compared to saline controls. This is in contrast to previous sexual conditioning work with cocaine. Levens and Akins (2004) reported that male quail that received chronic pre-exposure to cocaine displayed greater conditioned approach relative to paired saline or unpaired controls during sexual conditioning. One important methodological consideration is that Levens and Akins (2004) used a 10-day withdrawal period whereas a 31-day withdrawal period was used in the current study. The longer withdrawal period used in the current study may have allowed the neural circuitry affected by methamphetamine to recover such that no changes in sexual conditioning were evident after this period of time. Previous research with rodents has shown that there may be reduced dopamine receptor binding in reward-relevant mesolimbic brain regions 24h following methamphetamine but not 7 or 30 days after methamphetamine administration (Stefanski et al. 1999, 2002). Although tentative, this may suggest that methamphetamine has a transient effect on the reward circuitry and this may allow behaviors that work via that circuitry to recover after a relatively long period of drug abstinence.

Another major methodological difference between Levens and Akins (2004) and the current findings was the use of cocaine versus methamphetamine. Although cocaine and methamphetamine are both psychostimulants that enhance extracellular neurotransmitter concentrations (e.g., dopamine and glutamate), they are known to have different pharmacological mechanisms of action and toxicity. For example, cocaine blocks the reuptake of dopamine at the dopamine transporter whereas methamphetamine sequesters dopamine from vesicles, while blocking reuptake of dopamine and promoting reverse transport at the dopamine transporter (Farnsworth et al., 2009; Fleckenstein et al., 2007). Methamphetamine may also have more widespread effects on glutamatergic and

GABAergic systems that may contribute to enhanced neurotoxicity (Fleckenstein et al., 2007). Therefore, it is possible that cocaine and methamphetamine have different effects on the neural circuitry that resulted in different behavioral outcomes between the two studies.

During copulatory opportunities in the current experiment, quail that previously received 3.0 mg/kg of methamphetamine made fewer CCMs relative to quail that received saline. This finding is in agreement with previous studies that have shown exposure to methamphetamine impaired copulation in rats. Saito and colleagues (1991) found decreased sexual performance in male rats following an acute dose of methamphetamine (4 mg/kg IP) and also following repeated once-weekly administration of methamphetamine (1 mg/kg IP). Similar impairments in copulatory performance in response to methamphetamine have also been reported in a more recent study with rodents (Frohman et al. 2010).

In the aforementioned studies, copulatory performance was tested shortly after acute or subchronic administration of methamphetamine whereas in the current study, copulatory performance was tested following a 31-day withdrawal period. The results of the current study are the first to suggest that impairments in sexual performance induced by prior methamphetamine exposure may be relatively long lasting. One tentative interpretation of this finding is that deficits in copulatory performance in quail may potentially be masked early during withdrawal due to compensation but may manifest later on. Previous research with humans has suggested that discontinuation of chronic stimulant use may result in a persistent inability to experience pleasure from activities that were once enjoyable (i.e., anhedonia; Leventhal et al. 2008). Similar behavioral phenomena have been observed in animals withdrawn from chronic exposure to amphetamines and these changes in behavior appear to correspond with alterations in brain monoamine levels that persist into the withdrawal period (see Kitanaka et al. 2008 for review). Previous research has suggested that socially directed behaviors may be especially vulnerable to the deleterious effects of methamphetamine. Specifically, chronic intermittent methamphetamine (8 mg/kg once weekly for 16 weeks) has been shown to decrease social interaction in rats 49 days after cessation of drug treatment (Clemens et al. 2007).

In contrast to the current findings, Bolin and Akins (2009) failed to show an impairment of copulatory behavior induced by chronic pre-exposure to methamphetamine when they gave copulation tests 10 days after the last methamphetamine injection. One potential explanation for the difference between the two experiments is that Bolin and Akins (2009) gave copulatory tests 3–5 hours after visual access to a female. In the current study, copulatory tests were given either immediately after presentation of a CS (paired) or 2–3 hours before CS presentation (unpaired). Although speculative, it is possible that this procedural difference could have resulted in different motivational levels, thereby resulting in differences in copulation. Another explanation may be that the extended withdrawal from methamphetamine may have allowed enough time for reorganization of neural circuitry and/or persistent alterations in neurotransmitter release to manifest behaviorally as a deficit in copulatory performance. Previous autoradiography research with quail has demonstrated decreases in receptor binding in sexually relevant brain regions in the absence of changes in mesolimbic reward circuitry (Balthazart and Ball, 1989). It may therefore be reasonable to suggest that the lack of effect of methamphetamine on sexual conditioning in the current study may be linked to a lack of changes (or recovery of function) in mesolimbic pathways and that methamphetamine may have deleterious effects on the neural circuitry that directly regulate sexual behavior to a greater extent compared to mesolimbic circuits. It remains unclear as to what mechanism(s) may have caused deficits in copulatory performance that resulted from chronic exposure to methamphetamine following 31 days of withdrawal. Future research will be needed to further explore this phenomenon and to determine the

mechanisms that underlie the changes in copulatory behavior that appear during withdrawal from methamphetamine in male Japanese quail.

4.1. Conclusions

In sum, the findings of the locomotor activity phase of the current study suggest that repeated administration of methamphetamine may result in tolerance or stereotypy in male Japanese quail. However, studies that investigate the pharmacokinetics of methamphetamine in avian species are unavailable so this interpretation should be considered tentative. The current findings suggest that chronic pre-exposure to methamphetamine does not alter sexual conditioning as found in previous sexual conditioning studies with cocaine. Methamphetamine does appear to impair sexual performance and this impairment may manifest and/or persist long after discontinuation of methamphetamine treatment. Although speculative, the current findings may suggest that there are differential rates of onset or recovery in drug-induced changes in the neural circuitry that mediates reward relative to the circuitry that mediates consummatory behavior following chronic methamphetamine exposure. Future research is needed to elucidate whether this phenomenon is mediated through separate neural circuits and how temporal disparity in the recovery of the neural circuitry may arise following withdrawal from chronic drug administration.

Acknowledgments

This work was submitted in partial fulfillment of a Master's degree requirement for B.L. Bolin. It was supported by NIDA grant DA00508 awarded to C.K. Akins and NIDA training grant T32 DA007304 entitled "Research Training in Drug Abuse Behavior" awarded to B.L. Bolin (T. Garrity, Program Director). We thank Luke Cornett, Grant Harris, and Katie Perkins for their help with data collection and animal care. We also thank NIDA for providing the methamphetamine used in this study.

References

- Ago Y, Arikawa S, Yata M, Yano K, Abe M, Takuma K, Matsuda T. Role of prefrontal dopaminergic neurotransmission in glucocorticoid receptor-mediated modulation of methamphetamine-induced hyperactivity. *Synapse*. 2009; 63:7–14. [PubMed: 18925659]
- Balthazart J, Ball GF. Effects of the noradrenergic neurotoxin DSP-4 on luteinizing hormone levels, catecholamine concentrations, alpha 2-adrenergic receptor binding, and aromatase activity in the brain of the Japanese quail. *Brain Res*. 1989; 492:163–175. [PubMed: 2568873]
- Barr AM, Fiorino DF, Phillips AG. Effects of withdrawal from an escalating dose schedule of d-amphetamine on sexual behavior in the male rat. *Pharmacol Biochem Behav*. 1999; 64:597–604. [PubMed: 10548277]
- Bolin BL, Akins CK. Methamphetamine impairs sexual motivation but not sexual performance in male Japanese quail. *Exp Clin Psychopharmacol*. 2009; 17:10–20. [PubMed: 19186930]
- Castagna C, Ball GF, Balthazart J. Effects of dopamine agonists on appetitive and consummatory male sexual behavior in Japanese quail. *Pharmacol Biochem Behav*. 1997; 58:403–414. [PubMed: 9300599]
- Clemens KJ, Cornish JL, Hunt GE, McGregor IS. Repeated weekly exposure to MDMA, methamphetamine or their combination: long-term behavioural and neurochemical effects in rats. *Drug Alcohol Depend*. 2007; 86:183–190. [PubMed: 16884865]
- Domjan M, Hall S. Determinants of social proximity in Japanese quail (*Coturnix coturnix japonica*): male behavior. *J Comp Psychol*. 1986; 100:59–67. [PubMed: 3754503]
- Domjan M, Lyons R, North NC, Bruell J. Sexual Pavlovian conditioned approach behavior in male Japanese quail (*Coturnix coturnix japonica*). *J Comp Psychol*. 1986; 100:413–421. [PubMed: 3802785]
- Farnsworth SJ, Volz TJ, Hanson GR, Fleckenstein AE. Cocaine alters vesicular dopamine sequestration and potassium-stimulated dopamine release: the role of D2 receptor activation. *J Pharmacol Exp Ther*. 2009; 328:807–812. [PubMed: 19038779]

- Fleckenstein AE, Volz TJ, Riddle EL, Gibb JW, Hanson GR. New insights into the mechanism of action of amphetamines. *Annu Rev Pharmacol Toxicol*. 2007; 47:681–698. [PubMed: 17209801]
- Frohman KS, Pitchers KK, Balfour ME, Coolen LM. Mixing pleasures: review of the effects of drugs on sex behavior in humans and animal models. *Horm Behav*. 2010; 58:149–162. [PubMed: 20004662]
- Gentry WB, Ghafoor AU, Wessinger WD, Laurenzana EM, Hendrickson HP, Owens SM. (+)-Methamphetamine-induced spontaneous behavior in rats depends on route of (+)METH administration. *Pharmacol Biochem Behav*. 2004; 79:751–760. [PubMed: 15582684]
- Holder MK, Hadjimarkou MM, Zup SL, Blustein T, Benham RS, McCarthy MM, Mong JA. Methamphetamine facilitates female sexual behavior and enhances neuronal activation in the medial amygdala and ventromedial nucleus of the hypothalamus. *Psychoneuroendocrinology*. 2010; 35:197–208. [PubMed: 19589643]
- Holder MK, Mong JA. Methamphetamine enhances paced mating behaviors and neuroplasticity in the medial amygdala of female rats. *Horm Behav*. 2010; 58:519–525. [PubMed: 20399784]
- Kitanaka N, Kitanaka J, Takemura M. Behavioral sensitization and alteration in monoamine metabolism in mice after single versus repeated methamphetamine administration. *Eur J Pharmacol*. 2003; 474:63–70. [PubMed: 12909196]
- Kitanaka J, Kitanaka N, Takemura M. Neurochemical consequences of dysphoric state during amphetamine withdrawal in animal models: a review. *Neurochem Res*. 2008; 33:204–219. [PubMed: 17605106]
- Levens N, Akins CK. Chronic cocaine pretreatment facilitates Pavlovian sexual conditioning in male Japanese quail. *Pharmacol Biochem Behav*. 2004; 79:451–457. [PubMed: 15582016]
- Leventhal AM, Kahler CW, Ray LA, Stone K, Young D, Chelminski I, Zimmerman M. Anhedonia and amotivation in psychiatric outpatients with fully remitted stimulant use disorder. *Am J Addict*. 2008; 17:218–223. [PubMed: 18463999]
- Melega WP, Williams AE, Schmitz DA, DiStefano EW, Cho AK. Pharmacokinetic and pharmacodynamic analysis of the actions of D-amphetamine and D-methamphetamine on the dopamine terminal. *J Pharmacol Exp Ther*. 1995; 274:90–96. [PubMed: 7616454]
- Milesi-Halle A, Hendrickson HP, Laurenzana EM, Gentry WB, Owens SM. Sex- and dose-dependency in the pharmacokinetics and pharmacodynamics of (+)-methamphetamine and its metabolite (+)-amphetamine in rats. *Toxicol Appl Pharmacol*. 2005; 209:203–213. [PubMed: 15916788]
- Peachey E, Rogers B, Brien JF, Maclean A, Rogers D. Measurement of acute and chronic behavioural effects of methamphetamine in the mouse. *Psychopharmacology (Berl)*. 1976; 48:271–275. [PubMed: 823582]
- Richfield EK, Young AB, Penney JB. Comparative distribution of dopamine D-1 and D-2 receptors in the basal ganglia of turtles, pigeons, rats, cats, and monkeys. *J Comp Neurol*. 1987; 262:446–463. [PubMed: 2958517]
- Rosine BJ, Levi Bolin B, Akins CK. Chronic preexposure to methylphenidate cross-sensitizes methamphetamine in male Japanese quail. *Behav Pharmacol*. 2009; 20:352–355. [PubMed: 19584711]
- Saito TR, Aoki S, Saito M, Amao H, Niwa T, Terada M, Sugiyama M, Takahashi KW. Effects of methamphetamine on copulatory behavior in male rats. *Jikken Dobutsu*. 1991; 40:447–452. [PubMed: 1748163]
- Schein MW, Diamond M, Carter CS. Sexual performance levels of male Japanese quail (*Coturnix coturnix japonica*). *Anim Behav*. 1972; 20:61–67.
- Segal DS, Kuczenski R. Human methamphetamine pharmacokinetics simulated in the rat: single daily intravenous administration reveals elements of sensitization and tolerance. *Neuropsychopharmacology*. 2006; 31:941–955. [PubMed: 16123749]
- Segal DS, Kuczenski R, O'Neil ML, Melega WP, Cho AK. Prolonged exposure of rats to intravenous methamphetamine: behavioral and neurochemical characterization. *Psychopharmacology (Berl)*. 2005; 180:501–512. [PubMed: 15959831]

- Semple SJ, Strathdee SA, Zians J, Patterson TL. Sexual risk behavior associated with co-administration of methamphetamine and other drugs in a sample of HIV-positive men who have sex with men. *Am J Addict*. 2009; 18:65–72. [PubMed: 19219667]
- Shuto T, Kuroiwa M, Hamamura M, Yabuuchi K, Shimazoe T, Watanabe S, Nishi A, Yamamoto T. Reversal of methamphetamine-induced behavioral sensitization by repeated administration of a dopamine D1 receptor agonist. *Neuropharmacology*. 2006; 50:991–997. [PubMed: 16563442]
- Stefanski R, Ladenheim B, Lee SH, Cadet JL, Goldberg SR. Neuroadaptations in the dopaminergic system after active self-administration but not after passive administration of methamphetamine. *Eur J Pharmacol*. 1999; 371:123–135. [PubMed: 10357249]
- Stefanski R, Lee SH, Yasar S, Cadet JL, Goldberg SR. Lack of persistent changes in the dopaminergic system of rats withdrawn from methamphetamine self-administration. *Eur J Pharmacol*. 2002; 439:59–68. [PubMed: 11937093]
- Volkow ND, Wang GJ, Fowler JS, Telang F, Jayne M, Wong C. Stimulant-induced enhanced sexual desire as a potential contributing factor in HIV transmission. *Am J Psychiatry*. 2007; 164:157–160. [PubMed: 17202559]

Highlights

- Chronic methamphetamine was followed by a 31-day withdrawal period
- Methamphetamine altered sexual performance but not sexual conditioning
- A relatively long withdrawal period may have contributed to the findings
- Implicates a dichotomy between motivation and consummatory behavior

Figure 1.
Diagram of the apparatus used during sexual conditioning trials.

Experimental Time Line **Figure 2.**

Visual representation of the time line of procedures used in the current study.

Figure 3.

Mean ($\pm$ SEM) photobeam breaks for quail that received saline, METH 3.0 mg/kg, or METH 5.6 mg/kg during the first (upper panel) and second (lower panel) 30 min of the 60 min session for Trial 1 and Trial 10. * indicates significant difference from Saline ($p < 0.05$); † indicates significant difference from METH 3.0 ($p < 0.05$); ** indicates significant difference between Trial 1 and Trial 10 ($p < 0.05$).

Figure 4. Mean ($\pm$ SEM) time spent in the CS zone during the CS period for paired (upper panel) and unpaired (lower panel) quail that received saline, METH 3.0 mg/kg, or METH 5.6 mg/kg.

Figure 5. Mean ($\pm$ *SEM*) number of CCMs for paired (upper panel) and unpaired (lower panel) quail that received saline, METH 3.0 mg/kg, or METH 5.6 mg/kg. * indicates significant difference from Saline ($p < 0.05$).