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Incubation of Methamphetamine but not Heroin Craving After Voluntary Abstinence in Male and Female Rats

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We recently introduced an animal model of incubation of methamphetamine craving after choice-based voluntary abstinence in male rats. Here we studied the generality of this phenomenon to (1) female rats, and (2) male and female rats with a history of heroin self-administration. We first trained rats to self-administer palatable food pellets for 6 days (6 h per day) for either methamphetamine (0.1 mg/kg/infusion) or heroin (0.1 mg/kg/infusion) for 12 days (6 h/day). We then assessed relapse to drug seeking under extinction conditions after 1 and 21 abstinence days. Between tests, the rats underwent either voluntary abstinence (achieved via a discrete choice procedure between drug and palatable food; 20 trials/day) or home-cage forced abstinence. We found no sex differences in methamphetamine self-administration or in the strong preference for the palatable food over methamphetamine during the choice-based voluntary abstinence. In both sexes, methamphetamine seeking in the relapse tests was higher after 21 days of either voluntary or forced abstinence than after 1 day (incubation of methamphetamine craving). We also found no sex differences in heroin self-administration or the strong preference for the palatable food over heroin during the choice-based voluntary abstinence. However, male and female rats with a history of heroin self-administration showed incubation of heroin craving after forced but not voluntary abstinence. Our results show that incubation of methamphetamine craving after voluntary abstinence generalizes to female rats. Unexpectedly, prolonged voluntary abstinence prevented the emergence of incubation of heroin craving in both sexes.
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INTRODUCTION

Relapse to heroin or methamphetamine use can occur after prolonged abstinence and is often precipitated by exposure to drug-associated cues (Hunt *et al*, 1971; O'Brien *et al*, 1992; Rawson *et al*, 2004; Wikler, 1973). Based on their clinical observations, Gawin and Kleber (1986) proposed that cue-induced cocaine craving increases during early abstinence and remains elevated for extended time periods. An analogous phenomenon termed 'incubation of drug craving' has been observed in rats and mice trained to self-administer cocaine (Grimm *et al*, 2001; Mead *et al*, 2007; Neisewander *et al*, 2000) and rats trained to self-administer alcohol (Bienkowski *et al*, 2004), nicotine (Abdolahi *et al*, 2010; Funk *et al*, 2016), heroin (Fanous *et al*, 2012; Shalev *et al*, 2001), and methamphetamine (Li *et al*, 2015; Shepard *et al*, 2004). In animal models, incubation of drug craving refers to the time-dependent increase in drug seeking during forced abstinence (Pickens *et al*, 2011; Wolf, 2016).

From an animal model-to-human translational perspective, a limitation of rodent incubation of drug-craving studies is that the abstinence period preceding the relapse episodes (operationally defined as the resumption of non-reinforced drug seeking during a period of abstinence) is experimenter-imposed or forced and achieved by removing the subjects from the drug self-administration environment (Lu *et al*, 2004; Venniro *et al*, 2016; Wolf, 2016). In contrast, abstinence in humans is often voluntary due to either the negative consequences of chronic drug use or the availability of competing alternative non-drug rewards (Epstein *et al*, 2006; Katz and Higgins, 2003); in the latter case, relapse vulnerability is high after loss of the alternative rewards (Heilig *et al*, 2016).

Based on the above-mentioned 'translational' considerations, we recently developed a rat model of incubation of methamphetamine craving after prolonged alternative reward choice-based voluntary abstinence (Caprioli *et al*, 2015a; Caprioli *et al*, 2016). Our model is based on studies showing that most rats strongly prefer the non-drug reward when given a mutually exclusive choice between cocaine or methamphetamine and palatable food (Cantin *et al*, 2010; Caprioli *et al*, 2015b; Lenoir *et al*, 2007). In our first study (Caprioli *et al*, 2015a), we trained food-sated male Sprague–Dawley rats to self-administer palatable food (6 sessions) and then to self-administer methamphetamine under two conditions: 12 sessions (9 h/day) or 50 sessions (3 h/day); we chose these training conditions based on the escalation or DSM-IV rodent addiction models (Ahmed and Koob, 1998; Deroche-Gamonet *et al*, 2004). We then assessed methamphetamine seeking in relapse tests after 1 or 21 abstinence days; during

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testing, lever presses led to contingent delivery of a light cue previously paired with methamphetamine infusions but not methamphetamine (extinction conditions). Between tests, the rats underwent voluntary abstinence for 19 days (achieved *via* a mutually exclusive discrete choice procedure between methamphetamine and the palatable food in which, on any given trial, the rats can earn the food or drug reward but not both; 20 trials/day). Under our voluntary abstinence procedure, most rats achieve complete methamphetamine abstinence during most of the choice sessions (ie, zero choices of methamphetamine infusions), whereas some rats continue to occasionally self-administer a small number of drug infusions during these sessions (Caprioli *et al*, 2015a; Caprioli *et al*, 2016; Caprioli *et al*, 2015b) (see Figure 2). We found that under both training conditions, methamphetamine seeking in the extinction tests was higher after 21 abstinence days than after 1 day, demonstrating incubation of drug craving after voluntary abstinence. We also found that 'incubated' drug seeking on voluntary abstinence day 21 was similar in magnitude to the incubated drug seeking observed on forced abstinence day 21 (Caprioli *et al*, 2015a).

The goal of our present study was to determine the generality of the phenomenon of incubation of drug craving after choice-based voluntary abstinence that we observed in male rats trained to self-administer methamphetamine. Many studies over the years have shown behavioral and mechanistic differences between opiate and psychostimulant drugs (Badiani *et al*, 2011; Ettenberg, 2009; Ettenberg *et al*, 1982; Mello and Negus, 1996). Therefore, we studied whether incubation of drug craving after voluntary abstinence generalizes to the opiate drug heroin. In addition, several studies have demonstrated sex differences in psychostimulant self-administration and relapse (Anker and Carroll, 2011; Carroll and Lynch, 2016; Carroll *et al*, 2004; Lynch *et al*, 2002), including incubation of cocaine craving after forced abstinence (Kerstetter *et al*, 2008) and cue-induced methamphetamine seeking (Cox *et al*, 2013). Therefore, we compared incubation of methamphetamine and heroin craving after voluntary (and forced) abstinence in male versus female rats.

MATERIALS AND METHODS

For subjects, drugs, intravenous surgery, apparatus, food pellets self-administration, drug self-administration, discrete-trials choice procedure, abstinence phase, and relapse tests, see Supplementary Material online.

Exp. 1: Incubation of Methamphetamine Craving in Males and Female Rats After Forced and Voluntary Abstinence

The goal of Exp. 1 was to compare incubation of methamphetamine craving after forced and voluntary abstinence between male and female Sprague–Dawley rats. We used four groups of rats (Supplementary Figure S1) that we trained and tested at the same time (voluntary abstinence: $n = 10$ males and 11 females; forced abstinence: $n = 10$ males and 10 females) in a mixed experimental design that included the between-subjects factors of Abstinence Condition (forced, voluntary) and Sex (male, female), and the within-subjects factor of Abstinence Day (1, 21). This experiment consisted of three experimental conditions: training phase, discrete choice test, and relapse tests.

Training. We first trained rats to self-administer palatable food pellets (6 sessions, 6 h/session; 5 pellets per reward delivery) and then trained them to self-administer methamphetamine (12 sessions, 6 h/session; 0.1 mg/kg/infusion per drug delivery).

Discrete choice tests. We determined food versus methamphetamine preference using mutually exclusive discrete choice after every three-consecutive drug self-administration sessions in all four groups (three-choice tests) and for 19 days in the voluntary abstinence groups.

Relapse tests. We tested the forced and voluntary abstinence male and female rats for methamphetamine seeking under extinction conditions on abstinence days 1 and 21. The duration of the test session was 30 min on day 1 and 2 h on day 21. The duration of the test session on day 1 was 30 min to minimize carryover effect of extinction learning, which may subsequently decrease drug seeking on day 21 testing.

Exp. 2: Incubation of Heroin Craving in Males and Female Rats After Forced and Voluntary Abstinence

The goal of this experiment was to determine whether incubation of drug craving after voluntary abstinence generalizes to the opiate drug heroin. We used four groups of rats (Supplementary Figure S1) that we trained and tested at the same time (voluntary abstinence: $n = 15$ males and 16 females; forced abstinence: $n = 11$ males and 15 females) in a mixed experimental design that included the between-subjects factors of Abstinence Condition (forced, voluntary) and Sex (male, female), and the within-subjects factor of Abstinence Day (1, 21). This experiment consisted of three experimental conditions training phase, discrete choice test, and the relapse test.

Training. We first trained rats to self-administer palatable food pellets (6 sessions, 6 h/session; 5 pellets per reward delivery) and then trained them to self-administer heroin (12 sessions, 6 h/session; 0.1 mg/kg/infusion per drug delivery).

Discrete choice tests. We determined food versus heroin using a mutually exclusive discrete choice procedure after every three-consecutive drug self-administration sessions in all four groups (three-choice tests) and for 19 days in the voluntary abstinence groups.

Relapse test. We tested forced and voluntary abstinence male and female rats for heroin seeking under extinction conditions on abstinence days 1 and 21. As in Exp. 1, the duration of the test session was 30 min on day 1 and 2 h on day 21.

Statistical Analyses

We analyzed the data with the statistical program SPSS using GLM module. We analyzed all methamphetamine and heroin data separately. For the training phase, we analyzed the data separately for food rewards and drug infusions with mixed ANOVA, using the within-subjects factor of Session and the between-subjects factor of Sex (male, female). Because of the differences in body weight between male and female rats, we converted the number of rewards for palatable food pellets using the following formula: (number of reward × gram of one palatable food pellet × 5)/1000)/(body weight/1000). For the choice tests, we analyzed the data with mixed ANOVA, using the between-subjects factor of Sex and the within-subjects factors of Choice session and Reward type (food, drug). For the relapse tests, we analyzed inactive and active lever presses during the sessions using mixed ANOVA with the between-subjects factors of Abstinence condition (forced, voluntary) and Sex (male, female), and the within-subjects factors of Abstinence day (1, 21) and Lever (inactive, active). As our multifactorial ANOVAs yielded multiple main and interaction effects, we only report significant effects that are critical for data interpretation (see Supplementary Table S1 for a complete reporting of the statistical analyses).

RESULTS

Exp. 1: Incubation of Methamphetamine Craving in Males and Female Rats After Forced and Voluntary Abstinence

Training. During training for food self-administration, female rats earned significantly more food rewards relative to their body weight than male rats, but this difference decreased over time (Figure 1b). We observed a significant main effect of Sex ($F_{1,39} = 17.5$, $p < 0.0001$) and an interaction between Session and Sex ($F_{5,195} = 3.1$, $p = 0.01$). During training for methamphetamine self-administration, the male and female rats escalated their methamphetamine intake over time with somewhat steeper escalation in males (Figure 1c). We observed a significant effect of Session ($F_{11,429} = 69.0$, $p < 0.0001$) and an interaction between Session and Sex ($F_{11,429} = 2.2$, $p = 0.01$). During the three discrete choice sessions, the male and female rats showed a strong preference for food, an effect that was somewhat stronger in males (Figure 1d). We observed a significant main effect of Reward type ($F_{1,39} = 226.8$, $p < 0.0001$), Sex ($F_{1,39} = 5.1$, $p = 0.03$), and an interaction between Reward type and Session ($F_{2,78} = 7.4$, $p = 0.001$).

Abstinence phase. During the 3-week abstinence phase, the male and female rats in the voluntary abstinence groups showed a strong preference for food, but this preference was somewhat lower for females (Figure 2a). We observed a significant effect of Reward type ($F_{1,19} = 854.1$, $p < 0.0001$) and an interaction between Reward type and Sex ($F_{1,19} = 7.4$, $p = 0.01$).

Relapse tests. Active lever presses during the tests were higher after 21 abstinence days than after 1day (Figure 3a), demonstrating incubation of methamphetamine craving after forced or voluntary abstinence in male and female rats. We observed a significant interaction between Abstinence day and Lever ($F_{1,37} = 72.6$, $p < 0.0001$), but no effects of Abstinence condition or Sex, or any interactions between these factors. In addition, analysis of the time course data of active lever presses of the entire 2 h session on day 21 showed a significant main effect of Session hour ($F_{1,37} = 120.4$, $p < 0.0001$), but no effects of Abstinence condition or Sex, or interactions between the factors. Finally, inactive lever presses were very low (mean responding of 1.0 ± 0.4 to 5.4 ± 2.0 in the different conditions) and did not differ between the abstinence days, the abstinence conditions, or the sexes (p-values > 0.05).

Exp. 2: Incubation of Heroin Craving in Males and Female Rats After Forced and Voluntary Abstinence

Training. As in Exp. 1, during food self-administration training, female rats earned significant more food rewards relative to their body weight than male rats, and this difference decreased over time (Figure 1e). We observed a significant effect of Sex ($F_{1,55} = 10.8$, $p = 0.002$) and an interaction between Session and Sex ($F_{2,275} = 5.1$, $p < 0.0001$). During training for heroin self-administration, both male and female rats increased their drug intake over time (Figure 1f). We observed a significant effect of Session ($F_{11,605} = 15.0$, $p < 0.0001$) but no effects of Sex or an interaction between the two factors (p-values > 0.1). During the three discrete choice sessions, both male and female rats showed a strong preference for food (Figure 1g). We observed a significant effect of Reward type ($F_{1,55} = 1002.2$, $p < 0.0001$) and an interaction between Reward type and Session ($F_{2,110} = 48.8$, $p < 0.0001$), but no significant effect of Sex or interactions between Sex and the other factors (p-values > 0.1).

Abstinence phase. During the 3-week abstinence phase, male and female rats in the voluntary abstinence groups showed a strong preference for food (Figure 2c). We observed a significant effect of Reward type ($F_{1,29} = 15606.5$, $p < 0.0001$) and an interaction between Reward type and Session ($F_{18,522} = 1.8$, $p = 0.019$).

Relapse tests. Active lever presses during the tests were higher after 21 abstinence days than after 1 day in the forced but not voluntary abstinence condition (Figure 3d), demonstrating incubation of heroin craving after forced but not voluntary abstinence in male and female rats. The statistical analysis included the between-subjects factors of Sex and Abstinence condition (forced, voluntary), and the within-subjects factors of Abstinence day (1, 21) and Lever (active, inactive). We observed a significant interaction between Abstinence day and Abstinence condition ($F_{1,53} = 10.8$, $p = 0.002$). In addition, analysis of the time course data of active lever presses of the entire 2 h session on day 21 showed significant main effects of Abstinence condition ($F_{1,53} = 11.7$, $p = 0.001$) and Session hour ($F_{1,53} = 73.0$, $p < 0.0001$). Finally, inactive lever presses were very low (mean responding of 1.3 ± 0.3 to 5.8 ± 1.1 in the different conditions) and did not differ between the abstinence days, the abstinence conditions, or the sexes (p-values > 0.05).

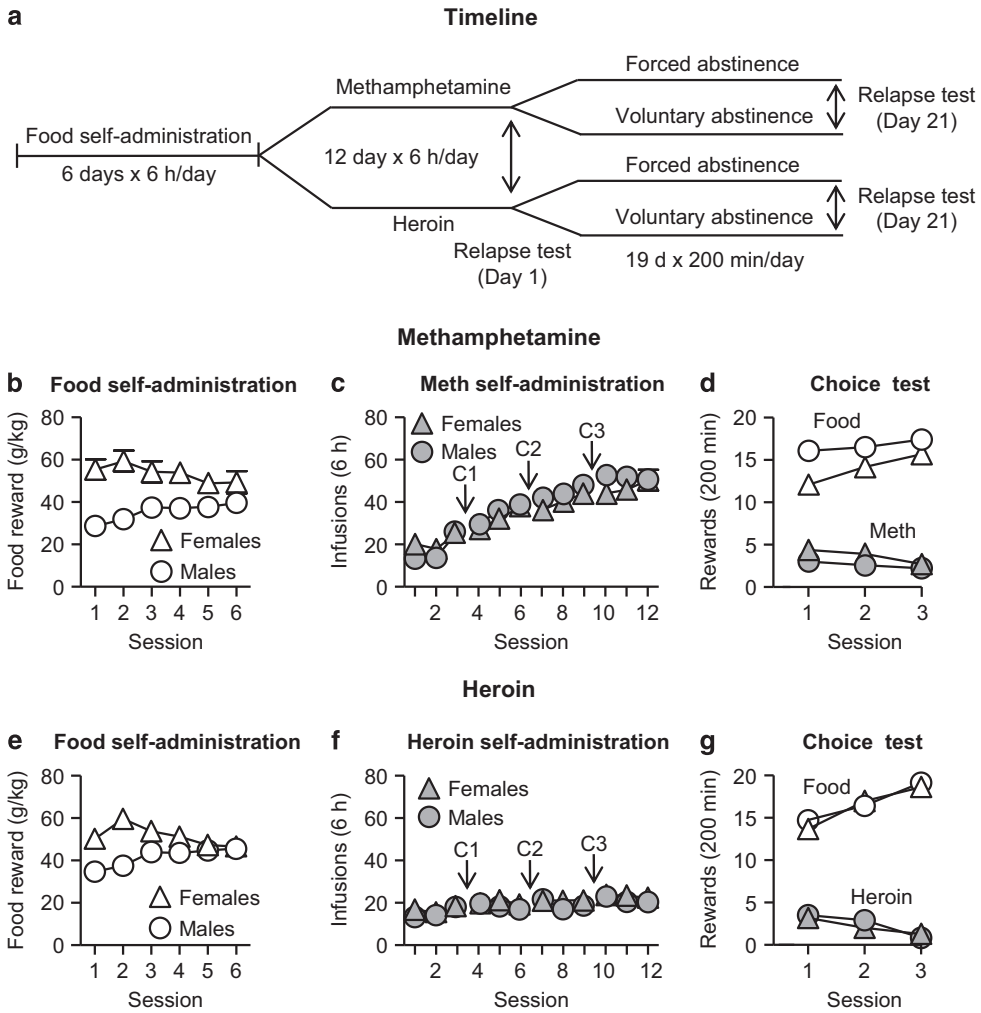

Figure 1 Experimental timeline and self-administration training for palatable food, methamphetamine, and heroin in male and female rats. (a) Timeline of the experiment. (b) Food self-administration for methamphetamine-trained rats: mean ± SEM amount of food rewards in g/kg during the 6 h sessions. (c) Methamphetamine self-administration: mean ± SEM number of methamphetamine infusions during the 6 h sessions. C, choice sessions. (d) Discrete choice tests: mean ± SEM food rewards and methamphetamine infusions earned during the three discrete choice sessions during training. (e) Food self-administration for heroin-trained rats: mean ± SEM amount of food rewards in g/kg during the 6 h sessions. (f) Heroin self-administration: mean ± SEM number of heroin infusions during the 6 h sessions. C, choice sessions. (g) Discrete choice tests: mean ± SEM food rewards and heroin infusions earned during the three discrete choice sessions during training. Methamphetamine: females, $n = 21$, males, $n = 20$; Heroin: females, $n = 31$, males, $n = 26$.

DISCUSSION

We recently introduced an animal model of incubation of methamphetamine craving after choice-based voluntary abstinence in male rats (Caprioli *et al*, 2015a). In the present study, we determined the generality of this phenomenon to female rats and to male and female rats with a history of heroin self-administration. There are two main findings in our study. The first is that we found no sex differences in incubation of methamphetamine craving after voluntary abstinence. The second is that choice-based voluntary abstinence prevented the emergence of incubation of heroin craving in both sexes. We also found no evidence for sex differences in methamphetamine or heroin self-administration, the strong preference for the palatable food over these drugs, and incubation of drug craving after forced abstinence. Finally, we found that after adjusting for body weight, female rats self-administered more palatable food than male rats, an observation in agreement with some studies (Klump *et al*, 2013; Reichelt *et al*, 2016) but not others (Cifani *et al*, 2012; Pickens *et al*, 2012).

Incubation of Methamphetamine Craving After Voluntary or Forced Abstinence In Male and Female Rats

In Exp. 1 we found no evidence for sex differences in methamphetamine self-administration and incubation of drug craving after voluntary or forced abstinence. This pattern of results contrasts with previous reports on sex differences in methamphetamine self-administration and relapse, showing that compared with male rats, female rats acquire methamphetamine self-administration faster, and show potentiated cue- and drug priming-induced reinstatement of methamphetamine seeking after extinction (Cox *et al*, 2013; Holtz *et al*, 2012; Reichel *et al*, 2012; Roth

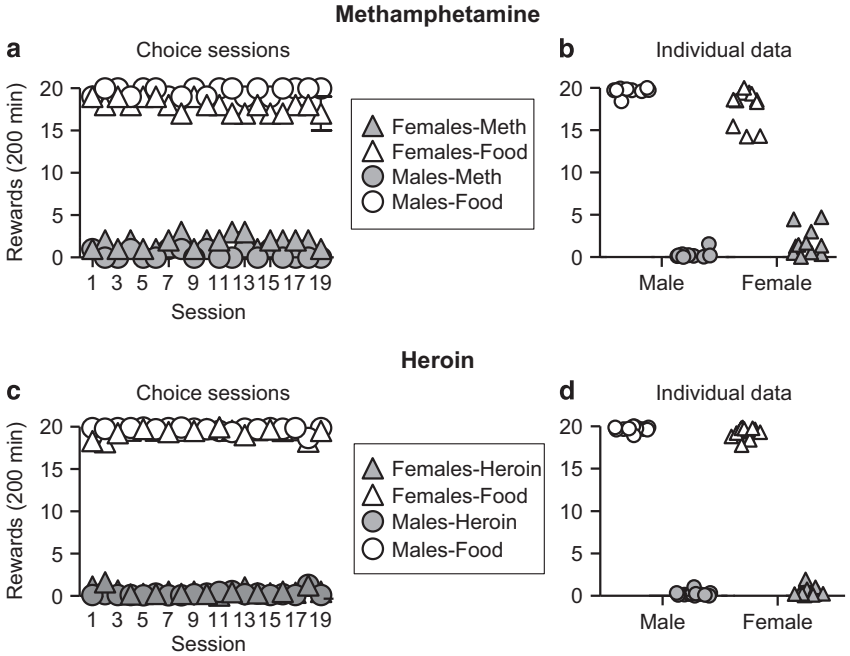

Figure 2 Voluntary abstinence. (a) Methamphetamine voluntary abstinence: mean ± SEM food rewards (5 pellets per reward delivery) and methamphetamine infusions earned during the 19 discrete choice sessions. (b) Individual data for methamphetamine voluntary abstinence: individual mean food rewards and methamphetamine infusions earned across the 19 discrete choice sessions. (c) Heroin voluntary abstinence: mean ± SEM food rewards (5 pellets per reward delivery) and heroin infusions earned during the 19 discrete choice sessions. (d) Individual data for heroin voluntary abstinence: individual mean food rewards and heroin infusions earned across the 19 discrete choice sessions. Methamphetamine: females, $n = 11$, males, $n = 10$; Heroin: females, $n = 16$, males, $n = 15$.

and Carroll, 2004). What might account for our results showing no sex differences in methamphetamine self-administration and relapse and the reported sex differences in the above-mentioned studies?

Regarding methamphetamine self-administration, we suspect that the negative results in our study versus the positive results of sex differences in the previous studies (Reichel *et al*, 2012; Roth and Carroll, 2004) are due to the use of higher unit dose in our study than in these studies. Indeed, there is evidence that sex differences in cocaine and nicotine self-administration are more apparent when low drug doses are used (Carroll *et al*, 2001; Donny *et al*, 2000; Lynch and Carroll, 1999). Regarding the methamphetamine unit dose in our study, we chose a high dose (0.1 mg/kg) based on our previous reports in male rats showing reliable self-administration and incubation of drug craving after forced abstinence with this dose (Caprioli *et al*, 2015a; Li *et al*, 2015; Shepard *et al*, 2004; Theberge *et al*, 2013).

Regarding relapse, our findings of the lack of sex differences in relapse to methamphetamine seeking are different from those reported using the classical reinstatement model (Shaham *et al*, 2003) of higher cue- and drug priming-induced reinstatement in female rats than in male rats (Cox *et al*, 2013; Reichel *et al*, 2012). In reconciling the differences between our results and those of these previous studies, an important consideration is that there is evidence that the mechanisms underlying reinstatement after extinction in the self-administration environment can be dissociable from those controlling drugs seeking during forced abstinence in the homecage without extinction training (Fuchs *et al*, 2006; Marchant *et al*, 2013). We speculate that extinction training, which causes neuroadaptations in brain areas critical for reinstatement of drug seeking like the nucleus accumbens (Knackstedt *et al*, 2010; Self *et al*, 2004), may recruit sex-specific mechanisms that increase the sensitivity of female rats to methamphetamine priming- and cue-induced reinstatement of drug seeking. Thus, sex differences in reinstatement of drug seeking after extinction may not generalize to abstinence-based models of drug relapse in which extinction procedures are not used.

Another finding in Exp. 1 was the lack of sex differences in the strong preference for palatable food over methamphetamine. There are no published studies on sex differences between methamphetamine and palatable food. However, our results are different from results reported in two studies showing that a higher proportion of female rats prefer cocaine over food (Kerstetter *et al*, 2012; Perry *et al*, 2013). Comparison across choice studies using different drugs and different food types should be done with caution. However, we suspect that the strong preference for food and lack of sex differences in our study are due to the use of a high reward magnitude (five pellets per reward delivery) and a highly palatable food that is preferred over all other commercially available 45 mg pellet types of different food compositions and flavors (Calu *et al*, 2014), which is more resistant to punishment-induced suppression of operant responding than methamphetamine (Krasnova *et al*, 2014). In contrast, in the above-mentioned cocaine choice studies, investigators have used pellet types that were less preferred than our pellet type and lever presses led to the delivery of a single food pellet, allowing for the detection of both individual

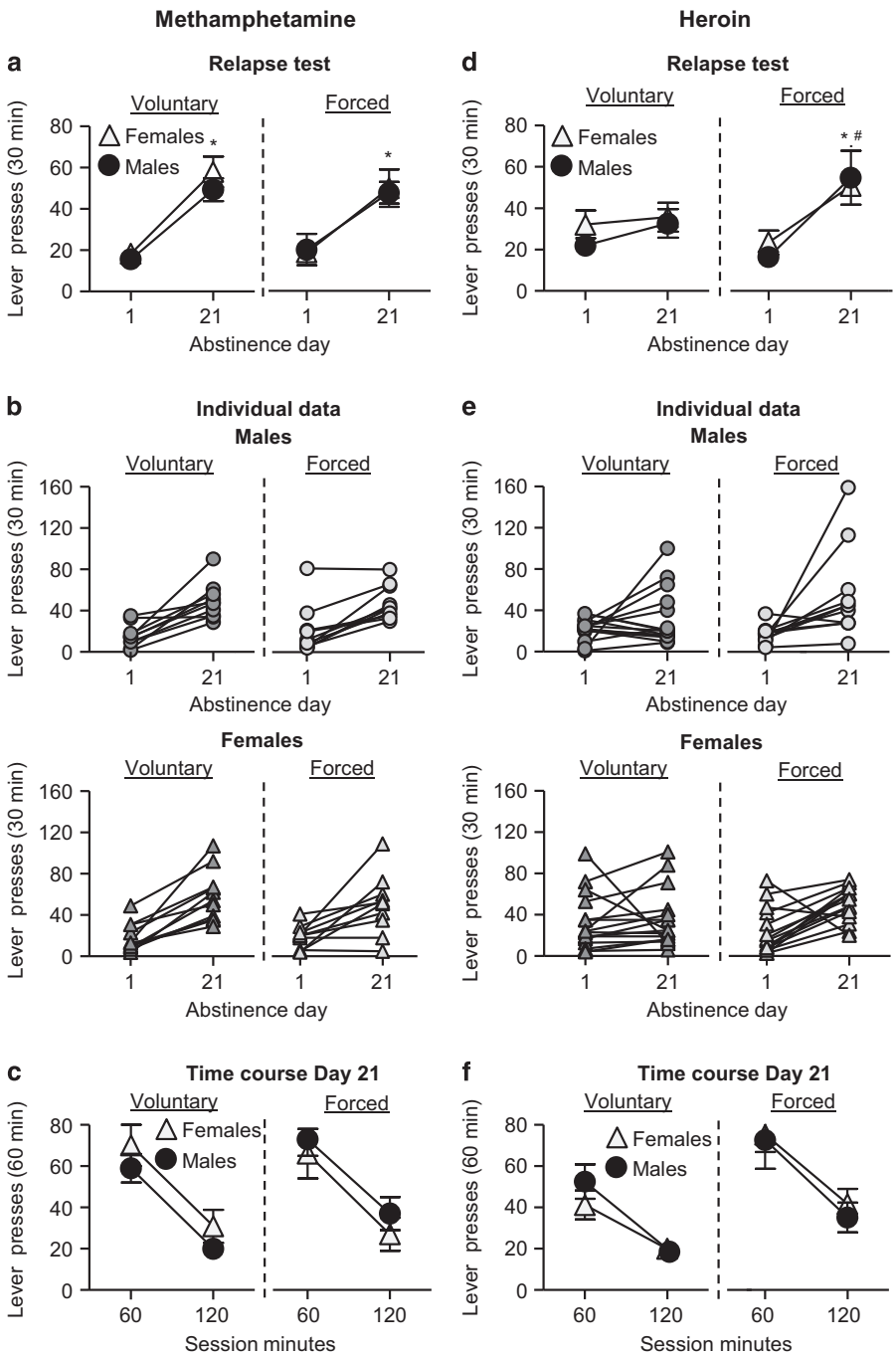

Figure 3 Relapse tests (a) Methamphetamine seeking on abstinence days 1 and 21 (group data): mean ± SEM lever presses during the 30 min relapse tests. (b) Methamphetamine: individual data: individual lever presses during the tests. (c) Methamphetamine seeking on day 21 (time course, group data): mean ± SEM lever presses at each hour of the test session on day 21. (d) Heroin seeking on abstinence on days 1 and 21 (group data): mean ± SEM lever presses during the 30 min relapse tests. (e) Heroin: individual data: individual lever presses during the tests. (f) Heroin seeking on day 21 (time course, group data): mean ± SEM lever presses at each hour of the test session on day 21. *Different from day 1, $p < 0.05$; #Different from voluntary abstinence day 21, $p < 0.05$. Methamphetamine: voluntary abstinence: $n = 10$ males and 11 females; forced abstinence: $n = 10$ males and 10 females. Heroin: voluntary abstinence: $n = 15$ males and 16 females; forced abstinence: $n = 11$ males and 15 females.

differences and sex differences in cocaine versus food choice (Kerstetter *et al*, 2012; Perry *et al*, 2013).

Finally, the lack of sex differences in incubation of methamphetamine craving after forced abstinence were not predicted based on a comprehensive study of Kerstetter *et al* (2008) showing sex differences in incubation of cocaine craving. An important consideration regarding these discrepant results is that recent evidence indicates mechanistic differences in incubation of cocaine versus methamphetamine craving (Li *et al*, 2015; Scheyer *et al*, 2016). Thus, sex differences in brain function that impact mechanisms of

incubation of cocaine craving may not generalize to incubation of methamphetamine craving.

Incubation of Heroin Craving After Forced But Not Voluntary Abstinence in Males and Females

In Exp. 2, we observed robust incubation of heroin craving after forced but not voluntary abstinence in both sexes. These results show an unexpected dissociation between methamphetamine and heroin for incubation of drug craving after voluntary abstinence.

We propose that this dissociation should be considered within the context of a large body of studies, suggesting that opiate reward and relapse, as assessed in rodent and monkey models, are mediated at least in part by behavioral and neurobiological mechanisms that are distinct from those of psychostimulants (Badiani *et al*, 2011; Ettenberg, 2009). Particularly relevant here is the demonstration that opioid receptors are critical for heroin but not psychostimulant reward (Badiani *et al*, 2011; Ettenberg *et al*, 1982; Mello and Negus, 1996). Endogenous opioid systems and opioid receptors have a critical role in palatable food reward (Baldo, 2016; Gosnell and Levine, 2009; Kelley and Berridge, 2002; Levine *et al*, 1985; Mena *et al*, 2013; Pecina and Berridge, 2005). Thus, we speculate that in our mutually exclusive choice-based voluntary abstinence procedure, the palatable food pellets act as a substitute for heroin (but not methamphetamine) in a manner akin to agonist substitution therapy (Dole, 1988; Dole and Nyswander, 1965), leading to decreased heroin seeking 1 day after the removal of the food substitute.

An alternative explanation of our data is that inhibition of incubation of heroin craving was due to some heroin exposure during voluntary abstinence (mean of 0.1 and 0.5 infusions per day or 0.01 and 0.05 mg/kg/day for males and females, respectively). Although we cannot rule out this possibility, we believe it is unlikely, because we found no correlation between drug intake during the choice sessions and active lever presses on the relapse test on day 21 (Pearson $r = 0.09$).

Two other findings in Exp. 2 were the lack of sex differences in heroin self-administration or the strong preference for the palatable food over heroin during the choice-based voluntary abstinence phase. The lack of sex differences in heroin self-administration in our study agrees with those from an early study, demonstrating similar heroin self-administration in males and females over a range of unit doses (0.06125 to 0.05 mg/kg/infusion) (Stewart *et al*, 1996). However, our results and those of Stewart *et al* (1996) are different from those of two other studies, showing that female rats acquired morphine and heroin self-administration faster than males (Cicero *et al*, 2003; Lynch and Carroll, 1999). A tentative conclusion from our study and these previous studies is that it has not been established that sex differences have a major role in heroin reward and relapse in rat models.

Finally, another finding in our study is the lack of sex differences in the strong preference for palatable food over heroin. At present, there are no published studies on sex differences in choice between heroin and food in animal models. However, our findings are consistent with previous reports of strong preference for a saccharin solution over heroin in male rats (Lenoir *et al*, 2013; Madsen and Ahmed, 2015; Vandaele *et al*, 2016).

Clinical Implications and Future Research Directions

In human addicts, abstinence is often voluntary due to the availability of alternative non-drug rewards (Epstein and Preston, 2003; Marlatt, 1996). This is exemplified in contingency management treatment where non-drug rewards (eg, monetary vouchers) given in exchange for being drug free (verified by drug testing) maintains prolonged abstinence (Higgins *et al*, 2004; Preston *et al*, 2002; Stitzer and Petry, 2006). However, when contingency management is discontinued, most addicts relapse to drug use (Roll, 2007; Silverman *et al*, 2012). As argued elsewhere, our rat model of relapse/incubation after choice-based voluntary abstinence is analogous to the human condition of relapse to drug use after termination of contingency management (Caprioli *et al*, 2015a). Regarding the 'translational' value of our new model, the data on lack of sex differences in both the efficacy of our choice-based procedure in inhibiting drug self-administration and relapse after cessation of 'contingency management' agree with the lack of gender differences in the efficacy of contingency management or the high relapse rates after treatment cessation in humans (Epstein *et al*, 2009; Lussier *et al*, 2006; Roll *et al*, 2013).

Our results have implications for future studies on incubation of methamphetamine and heroin craving, considering the recent NIH mandate of including both males and females in preclinical biomedical studies. Specifically, we found no evidence for sex differences in incubation of methamphetamine or heroin craving. Therefore, we tentatively suggest that in studies on incubation of drug craving it may not be necessary to use sex as an independent variable and double the *n* per experimental condition. Instead, we suggest including both males and females (preferably equal number) in each experimental condition, as advocated by Joel and McCarthy (2016) for behavioral models in which sex differences are not observed. This suggestion also makes sense, as we have observed similar variability in the behavioral response of the male and female rats under the different experimental conditions (see Figures 1,2,3), confirming recent meta-analyses of rodent studies indicating similar variability in males and females in different behavioral tasks (Becker *et al*, 2016; Prendergast *et al*, 2014).

Finally, although our new animal model mimics some features of human choice-based abstinence, it does not incorporate an important aspect of human drug addiction: the social environment (Heilig *et al*, 2016). In our study and most previous incubation of drug-craving studies (Venniro *et al*, 2016), the male and female rats were socially isolated. Thus, a question for future research is whether socially housing the rats, which previously shown to decrease incubation of cocaine craving after forced abstinence in male rats (Chauvet *et al*, 2012; Thiel *et al*, 2012), will also decrease incubation of drug craving after voluntary abstinence.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)