Archival Report

Effect of the Novel Positive Allosteric Modulator of Metabotropic Glutamate Receptor 2 AZD8529 on Incubation of Methamphetamine Craving After Prolonged Voluntary Abstinence in a Rat Model

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ABSTRACT

BACKGROUND: Cue-induced methamphetamine craving increases after prolonged forced (experimenter-imposed) abstinence from the drug (incubation of methamphetamine craving). Here, we determined whether this incubation phenomenon would occur under conditions that promote voluntary (self-imposed) abstinence. We also determined the effect of the novel metabotropic glutamate receptor 2 positive allosteric modulator, AZD8529, on incubation of methamphetamine craving after forced or voluntary abstinence.

METHODS: We trained rats to self-administer palatable food (6 sessions) and then to self-administer methamphetamine under two conditions: 12 sessions (9 hours/day) or 50 sessions (3 hours/day). We then assessed cue-induced methamphetamine seeking in extinction tests after 1 or 21 abstinence days. Between tests, the rats underwent either forced abstinence (no access to the food- or drug-paired levers) or voluntary abstinence (achieved via a discrete choice procedure between methamphetamine and palatable food; 20 trials per day) for 19 days. We also determined the effect of subcutaneous injections of AZD8529 (20 and 40 mg/kg) on cue-induced methamphetamine seeking 1 day or 21 days after forced or voluntary abstinence.

RESULTS: Under both training and abstinence conditions, cue-induced methamphetamine seeking in the extinction tests was higher after 21 abstinence days than after 1 day (incubation of methamphetamine craving). AZD8529 decreased cue-induced methamphetamine seeking on day 21 but not day 1 of forced or voluntary abstinence.

CONCLUSIONS: We introduce a novel animal model to study incubation of drug craving and cue-induced drug seeking after prolonged voluntary abstinence, mimicking the human condition of relapse after successful contingency management treatment. Our data suggest that positive allosteric modulators of metabotropic glutamate receptor 2 should be considered for relapse prevention.

Keywords: Abstinence, Addiction models, Discrete choice, Extended access, Glutamate, Incubation of drug craving, mGluR2/3, Palatable food, Positive allosteric modulator, Psychostimulants, Relapse, Self-administration

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Methamphetamine addiction is characterized by high relapse rates (1–3). Human drug relapse is often precipitated by exposure to drug-associated cues that provoke drug craving (4), now a formal criterion in the DSM-V (5). On the basis of clinical observations, Gawin and Kleber (6) proposed that cue-induced cocaine craving progressively increases during early abstinence and remains elevated for extended time periods. An analogous phenomenon, termed incubation of drug craving has been observed in rats trained to self-administer different drugs of abuse (7–11), including methamphetamine (12). In rodent models, incubation of drug craving refers to the time-dependent increases in cue-induced drug seeking during abstinence (13). Subsequent studies showed that incubation of drug craving also occurs in humans addicted to nicotine,

alcohol, and methamphetamine (14–16). These findings provide support for the translational potential of using rodent incubation models to identify pharmacologic targets for relapse prevention.

The abstinence period preceding relapse testing in studies using animal models is typically experimenter-imposed or forced; this is achieved either by removal of the laboratory animal from the drug self-administration environment or through extinction training (17,18). However, in humans, abstinence is often voluntary (self-imposed) with drug available in the addict's environment but forgone in favor of other nonaddictive alternative rewards (19,20). Current animal models of relapse after voluntary abstinence rely on punishment-induced suppression of drug intake (21–26). These models, as

well as other relapse/reinstatement models (27,28), do not capture the choice-based suppression of drug intake due to the presence of alternative nondrug rewards more typical of addicts (29,30). Indeed, evidence from contingency management studies in humans show that the availability of nondrug rewards (e.g., monetary vouchers), given in exchange for clean urine samples, can maintain abstinence for many months (31,32). Other drug addiction treatment strategies are also derived from operant learning principles, including community reinforcement approach (33) and behavioral self-control training (34). Importantly, when contingency management discontinues, most addicts relapse to drug use (3,35).

Based on these considerations and results from recent studies showing that when given a mutually exclusive choice between cocaine or methamphetamine and palatable foods most rats prefer the nondrug rewards over these drugs (36–39), we introduce here a rat model of incubation of drug craving after prolonged voluntary abstinence. Voluntary abstinence is achieved using a mutually exclusive discrete-choice procedure in which the alternative reward is palatable food (37–39).

In the present study, we trained rats to self-administer palatable food pellets and then trained them to self-administer methamphetamine using two established procedures to model addiction: extended daily access drug self-administration procedure (40,41) and a variation of a long-term training procedure used to identify addicted rats based on DSM-IV criteria (42,43). Subsequently, we exposed the rats to 3 weeks of voluntary abstinence using our recently established mutually exclusive choice procedure in which most rats strongly prefer palatable food over methamphetamine (39). We then assessed cue-induced methamphetamine seeking in extinction tests performed on abstinence days 1 and 21. For comparison purposes, we assessed incubation of methamphetamine craving after forced (experimenter-imposed) abstinence, the established incubation of craving model (44).

A second goal of our study was to pharmacologically characterize incubation of methamphetamine craving after voluntary (and forced) abstinence. For this purpose, we used AZD8529, a new and highly selective positive allosteric modulator (PAM) of metabotropic glutamate receptor 2 (mGluR2) (45), that was recently shown to inhibit cue- and drug-induced reinstatement of nicotine seeking in squirrel monkeys (46). mGluR2 PAMs bind to an allosteric site of the receptor and selectively activate these receptors in the presence of glutamate (47,48). mGluR2s are expressed primarily on presynaptic glutamate neurons and their activation by PAMs or agonists like LY379268 decreases evoked glutamate release (49). However, from a medication development perspective, there are limitations with LY379268 and related mGluR2/3 orthosteric agonists (see Discussion) that have led to the development of selective mGluR2 PAMs like AZD8529 (50,51).

METHODS AND MATERIALS

See supplement 1 for details on subjects, drugs, intravenous surgery, apparatus, procedures, abstinence phase, extinction tests, and statistical analyses.

Experiment 1: Incubation of Methamphetamine Craving After Short-Term Extended Daily Drug Access

We compared incubation of methamphetamine craving after forced [the established model (7,52)] and voluntary abstinence under extended daily access training conditions (22,53) (Figure 1). We used two groups of rats (n = 9–12 per group) in a mixed experimental design that included the between-subjects factor of abstinence condition (forced, voluntary) and the within-subjects factor of abstinence day (days 1 and 21). The experiment consisted of three phases: training phase, discrete choice tests, and the relapse test.

Training. We first trained the rats (n = 21) to self-administer palatable food pellets (six sessions, 9 hours/session; five pellets per reward delivery) and then trained them to self-administer methamphetamine (9 hours/session; 12 sessions; .1 mg/kg/infusion per reward delivery).

Discrete Choice Tests. We determined food versus methamphetamine choice after every three consecutive drug self-administration sessions in both groups (three choice tests) and then for 19 days in the voluntary abstinence group.

Relapse (Incubation) Test. We tested the forced and voluntary abstinence rats for cue-induced methamphetamine seeking under extinction conditions on abstinence days 1 and 21. The extinction test sessions were 30 minutes to minimize carryover effect and extinction learning/experience during day 1 testing, which may decrease incubated cue-induced drug seeking during day 21 testing.

Experiment 2: Incubation of Methamphetamine Craving After a Long-Term Limited Daily Drug Access

We compared incubation of methamphetamine craving after forced or voluntary abstinence following a drug-training procedure (50 sessions for 3 hours/day) that was based on the procedure used in the addiction model of Deroche-Gamonet *et al.* (42), Piazza and Deroche-Gamonet (43), and Deroche-Gamonet and Piazza (54) (Figure 2). We used two groups of rats (n = 8–9 per group) in a mixed experimental design that included the between-subjects factor of abstinence condition (forced, voluntary) and the within-subjects factor of abstinence day (days 1 and 21). The experiment consisted of three phases: training phase, discrete choice tests, and the relapse test.

Training. We first trained the rats (n = 17) to self-administer palatable food pellets (6 days, 3 hours/day; five pellets per reward delivery) and then trained them to self-administer methamphetamine (.1 mg/kg/infusion) for 3 hours/day for 50 days (5 training days per week).

Discrete Choice Tests. We determined food versus methamphetamine choice in both groups after every five consecutive methamphetamine self-administration sessions (nine choice tests) and then for 19 days in the voluntary abstinence

group. We gave the rats a day off after each choice day during training.

Relapse Test. We tested the forced and voluntary abstinence groups for cue-induced methamphetamine seeking under extinction conditions (30 min test) on abstinence days 1 and 21.

Experiment 3: Effect of AZD8529 on Incubation of Methamphetamine Craving After Forced or Voluntary Abstinence

We determined the effect of systemic injections of vehicle or AZD8529 (20 or 40 mg/kg, subcutaneous) on incubation of methamphetamine craving (Figure 3). We used six groups of rats (forced abstinence: $n = 8$–9; voluntary abstinence: $n = 14$–16) in a mixed experimental design that included the between-subjects factor of abstinence condition (forced, voluntary), AZD8529 dose (0, 20, 40 mg/kg), and the within-subjects factor of abstinence day (days 1 and 21). The experiment consisted of three phases: training phase, discrete choice tests, and the relapse test.

Training. We first trained the rats to self-administer palatable food pellets (6 days, 9 hours/day; five pellets per reward delivery) and then trained them to self-administer methamphetamine (9 hours/session; 12 sessions; .1 mg/kg/infusion per reward delivery).

Discrete Choice Tests. We determined food versus methamphetamine choice in both groups after every three consecutive drug self-administration sessions (three choice tests) and during 19 days for the voluntary abstinence group. We habituated the rats to the injection procedure for 2 days during this phase.

Relapse Test. We tested the forced and voluntary abstinence rats for cue-induced methamphetamine seeking under extinction conditions on abstinence days 1 and 21. The duration of the test session on day 1 was 30 minutes and on day 21 was 3 hours. We used a longer test session on day 21 to obtain more information on the behavioral effect of AZD8529 (3-hour pretreatment time) on incubated cue-induced methamphetamine seeking. As mentioned above, the duration of the test session on day 1 was 30 minutes to minimize carryover effect and extinction learning/experience during day 1 testing, which may decrease incubated cue-induced drug seeking during day 21 testing.

Experiment 4: Effect of AZD8529 on Food-Reinforced Responding

The goal of experiment 4 was to rule out that the effect of AZD8529 on cue-induced drug seeking on abstinence day 21 was due to motor deficits. For this purpose, we retrained rats ($n = 23$) previously used in experiment 3 to self-administer food pellets for 1 hour per day for 2 days. During the subsequent test session, we injected different groups of rats with vehicle or 20 or 40 mg/kg of AZD8529 ($n = 7$–8 per group) 3 hours before the session and measured pellet intake and lever presses during the test session.

RESULTS

Experiment 1: Incubation of Methamphetamine Craving After Short-Term Extended Daily Drug Access

The rats increased their food and methamphetamine intake over sessions (Figure 1A,B), and as in our previous study, extended access (9 hours/day) of methamphetamine led to strong escalation of drug intake (26,53,55). The repeated measures analysis of variance showed a significant effect of session for both food ($F_{5,100} = 3.5$, $p < .01$) and methamphetamine ($F_{11,220} = 37.5$, $p < .001$). During the three discrete choice sessions, the rats showed a strong preference for the food ($p < .01$, Figure 1C).

Abstinence Phase. During the 3-week abstinence period, the rats in the voluntary abstinence group showed a strong preference for food ($p < .01$), resulting in either no or minimal (one to two infusions per day) methamphetamine intake (Figure 1D).

Relapse (Extinction) Tests. Cue-induced methamphetamine seeking in the extinction tests was higher after 21 abstinence days than after 1 day, demonstrating incubation of methamphetamine craving after both forced and voluntary abstinence (Figure 1E). The statistical analysis of active lever presses included the between-subjects factor of abstinence condition (forced, voluntary), the within-subjects factor of abstinence day (days 1 and 21), and inactive lever presses as a covariate. This analysis showed a significant main effect of abstinence day ($F_{1,17} = 17.5$, $p < .01$) but not abstinence condition or an interaction between the two factors (p values $> .1$).

Correlations and Frequency Distribution. We examined the correlation (Pearson r) between the total number of methamphetamine infusions earned during the training sessions and the total number of lever presses during the extinction tests on abstinence day 21. We found no correlations between these two measures ($r = .14$, $p > .1$) (Figure 1G). In Figure 1F, we show the frequency distribution of extinction responding during testing on abstinence day 21 in reference to the mean of day 1 extinction responding.

Experiment 2: Incubation of Methamphetamine Craving After a Long-Term Limited Daily Drug Access

The number of food rewards earned remained stable during the six food training sessions ($p > .05$ for session effect). The rats increased the number of methamphetamine rewards earned during the first 10 training days (Figure 2A,B), as indicated by a significant effect of session ($F_{49,784} = 18.1$, $p < .01$). During the nine discrete choice sessions, the rats showed a strong preference for the food ($p < .01$, Figure 2C).

Abstinence Phase. During the 3-week abstinence period, the rats in the voluntary abstinence condition showed a strong preference for food, resulting in either no or minimal methamphetamine intake ($p < .01$, Figure 2D).
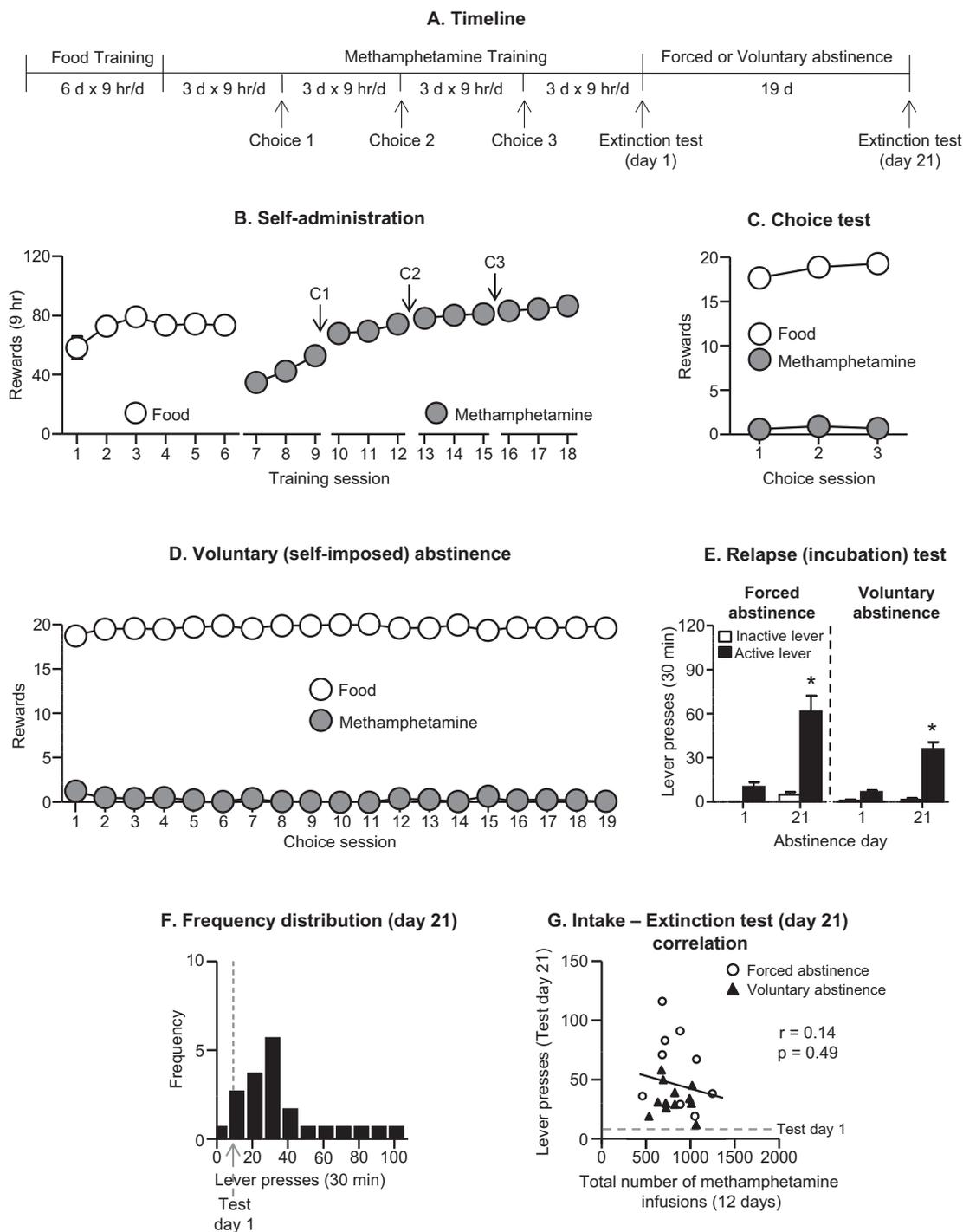
Figure 1. Incubation of methamphetamine craving after short-term extended daily access (9 hours/session, 12 sessions). **(A)** Timeline of the experiment. **(B)** Self-administration: Mean ± SEM number of food rewards (five palatable food pellets/reward delivery) or methamphetamine infusions (.1 mg/kg/infusion) during the 9-hour sessions. **(C)** Discrete choice tests: Mean ± SEM of food reward and methamphetamine infusions earned during the three discrete choice sessions that were performed during training (20 trials every 10 minutes). **(D)** Voluntary abstinence: Mean ± SEM of food reward and methamphetamine infusions earned during the 19 discrete choice sessions (20 trials every 10 minutes). **(E)** Relapse (extinction) tests: Mean ± SEM of lever presses on the previously active lever and on the inactive lever during the 30-minute extinction sessions. *Different from day 1 within each abstinence condition, $p < .01$ ($n = 9$–12 per group). **(F)** Frequency distribution: Frequency distribution of active lever presses during the 30-minute extinction test. **(G)** Extinction test correlation: The graph shows lack of correlation between the total number of methamphetamine infusions earned during the training sessions and the total number of lever presses during the extinction test on abstinence day 21. The dashed line in gray refers to the number of active lever presses on abstinence day 1.
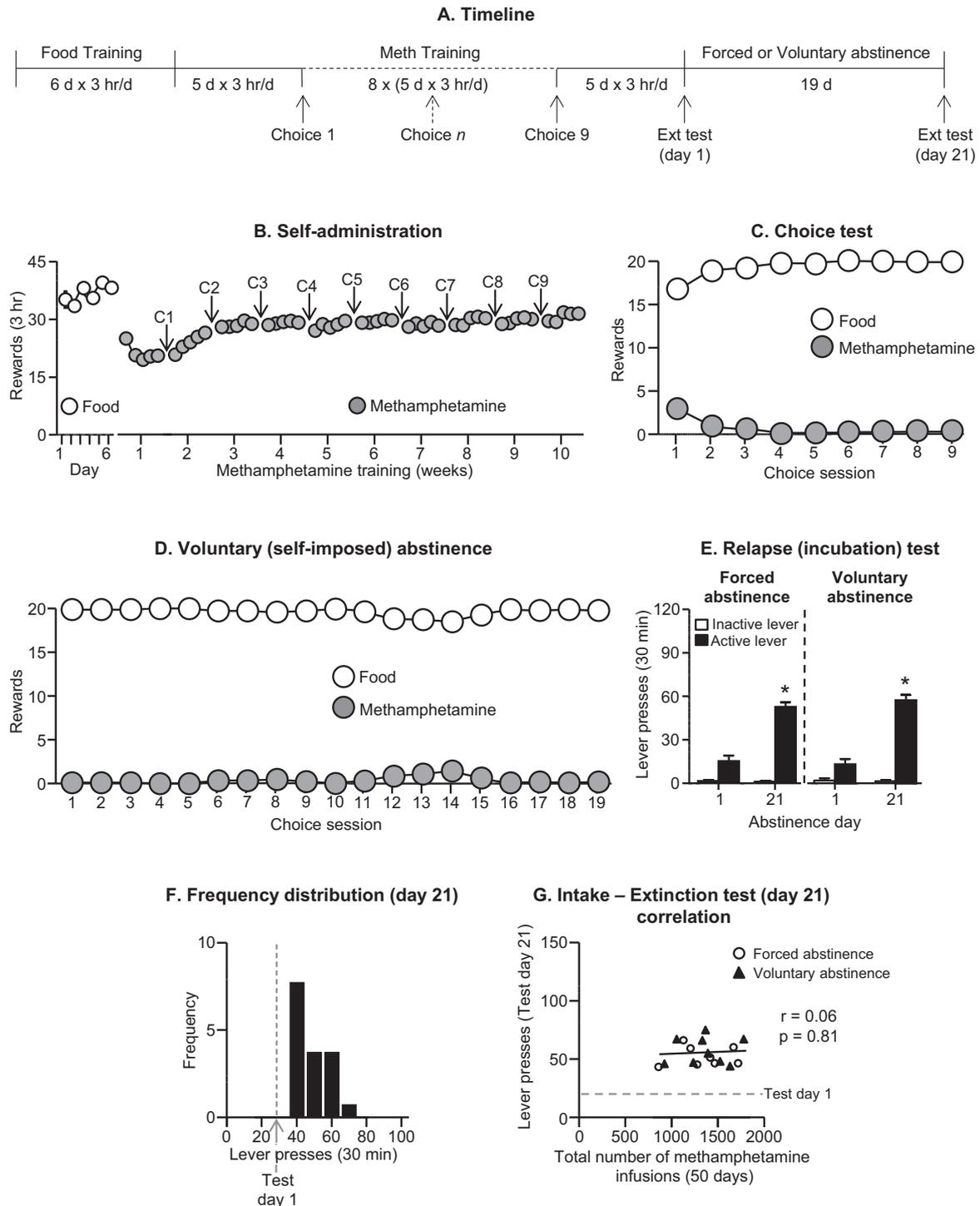
Figure 2. Incubation of methamphetamine craving after long-term limited daily access (3 hours/day, 50 sessions). **(A)** Timeline of the experiment. **(B)** Self-administration: Mean ± SEM number of food rewards or methamphetamine infusions during the 3-hour sessions. **(C)** Discrete choice tests: Mean ± SEM of food reward and methamphetamine infusions earned during the nine discrete choice sessions that were performed during training. **(D)** Voluntary abstinence: Mean ± SEM of food reward and methamphetamine infusions earned during the 19 discrete choice sessions. **(E)** Relapse (extinction) tests: Mean ± SEM of lever presses on the previously active lever and on the inactive lever during the 30-minute extinction sessions. *Different from day 1 within each abstinence condition, $p < .01$ ($n = 8$–9 per group). **(F)** Frequency distribution: Frequency distribution of active lever presses over 30-minute extinction test. **(G)** Extinction test correlation: The graph shows lack of correlation between the total number of methamphetamine infusions earned during the training sessions and the total number of lever presses during the extinction test on abstinence day 21. The dashed line in gray refers to the number of active lever presses on abstinence day 1. Ext, extinction.

Relapse (Extinction) Tests. Cue-induced methamphetamine seeking in the extinction tests was higher after 21 abstinence days than after 1 day after both forced and voluntary abstinence (Figure 2E). The statistical analysis of active lever presses included the between-subjects factor of abstinence condition, the within-subjects factor of abstinence day, and inactive lever presses as a covariate. This analysis showed a significant main effect of abstinence day ($F_{1,13}$ = 73.9, $p < .01$) but not abstinence condition or an interaction between the two factors (p values $> .1$).

Correlations and Frequency Distribution. We examined the correlation (Pearson r) between the total number of methamphetamine infusions earned during the training sessions and the total number of lever presses during the extinction tests on abstinence day 21. We found no correlations between these two measures ($r = .06$, $p > .1$) (Figure 2G). In Figure 2F, we show the frequency distribution of extinction responding during testing on abstinence day 21 in reference to the mean of day 1 extinction responding.

Experiment 3: Effect of AZD8529 on Incubation of Methamphetamine Craving After Forced or Voluntary Abstinence

Food and Methamphetamine Training. The rats modestly increased their food intake and as in experiment 1, strongly escalated their methamphetamine intake over days (Figure 3A,B). The repeated measures analysis of variance showed a significant effect of session for both food ($F_{5,355}$ = 7.3, $p < .01$) and methamphetamine ($F_{11,781}$ = 215.6, $p < .01$). During the three discrete choice tests, the rats showed a strong preference for the food ($p < .01$, Figure 3C).

Abstinence Phase. During the 3-week abstinence period, the rats in the voluntary abstinence condition showed a strong preference for food ($p < .01$), resulting in either no or minimal methamphetamine intake (Figure 3D).

Relapse (Extinction) Tests. Systemic injections of AZD8528 dose-dependently decreased cue-induced methamphetamine seeking in the extinction tests on abstinence day 21 but not day 1; this effect occurred after either forced or voluntary abstinence (Figure 3E). The statistical analysis of the 30-minute test data (the test duration on day 1) included the between-subjects factors of abstinence condition and AZD8529 dose, the within-subjects factor of abstinence day, and inactive lever presses as a covariate. This analysis showed a significant interaction between abstinence day and AZD8529 dose ($F_{2,64}$ = 4.7, $p < .01$), reflecting the selective effect of AZD8529 cue-induced methamphetamine seeking during late but not early abstinence. We also analyzed the data from the entire 3-hour day 21 test session using the factors of abstinence condition, AZD8529 dose, and session hour. This analysis showed significant effects of AZD8529 dose ($F_{2,66}$ = 6.5, $p < .01$) and session hour ($F_{2,132}$ = 90.4, $p < .01$) but no significant effects of abstinence condition or interactions between the three factors ($p > .1$). These statistical results indicate that AZD8529 decreased the response to the methamphetamine cues during the extinction tests but had no effect on within-session extinction learning (Figure 3E). Additional post hoc analyses showed that the effect of AZD8529 on lever responding on day 21 extinction test was significant at the 40 mg/kg dose but not the 20 mg/kg dose.

Experiment 4: Effect of AZD8529 on Food-Reinforced Responding

The goal of experiment 4 was to rule out that the effect of AZD8529 on the cue-induced drug seeking on abstinence day 21 was due to motor deficits or some other nonspecific performance impairments. We retrained rats ($n = 23$) previously used in experiment 3 to self-administer the palatable food pellets and determined the effect of AZD8529 (0, 20, or 40 mg/kg; $n = 7–8$ per dose) on ongoing food-reinforced responding. We found that systemic AZD8529 injections had no effect on food self-administration (Figure 4).

DISCUSSION

There are two main findings in our study. First, time-dependent increases in cue-induced methamphetamine seeking (incubation of methamphetamine craving) were reliably observed after prolonged periods of choice-based voluntary abstinence. This effect was observed under two different self-administration procedures that are widely used to model drug addiction: extended daily access drug self-administration procedure (40,41) and a long-term training procedure used to identify addicted rats based on DSM-IV criteria (42,43,54). Second, the mGluR2 PAM AZD8529 decreased incubated cue-induced methamphetamine seeking after prolonged voluntary or forced abstinence but had no effect on either nonincubated drug seeking during early abstinence or food-reinforced responding. The latter finding indicates that AZD8529 effects on cue-induced methamphetamine seeking are not due to motor deficits or some other nonspecific side effects. Finally, our results indicate that incubation of methamphetamine craving after voluntary abstinence is as robust as incubation after forced abstinence.

Implications for Animal Models of Drug Addiction and Relapse

The goal of our study was to develop a rat model to study incubation of drug craving that occurs after long-term voluntary abstinence. Our model was inspired in part by the recent findings of resurgence (reinstatement) of cocaine or alcohol seeking after extinction when an alternative food reward is removed (56,57). In the operant conditioning paradigm, resurgence refers to the recovery of an extinguished operant response after discontinuation of reinforcement of an alternative response (58–60).

We developed our animal model, because in current animal models of relapse, abstinence is experimenter-imposed (or forced) either via extinction training or by removing the rat from the self-administration chambers (17,18,27,28). Additionally, recent animal models of relapse that do rely on voluntary abstinence are based on punishment-induced suppression of drug intake (21–26), an aspect of drug addiction that is not captured in our choice-based relapse model.
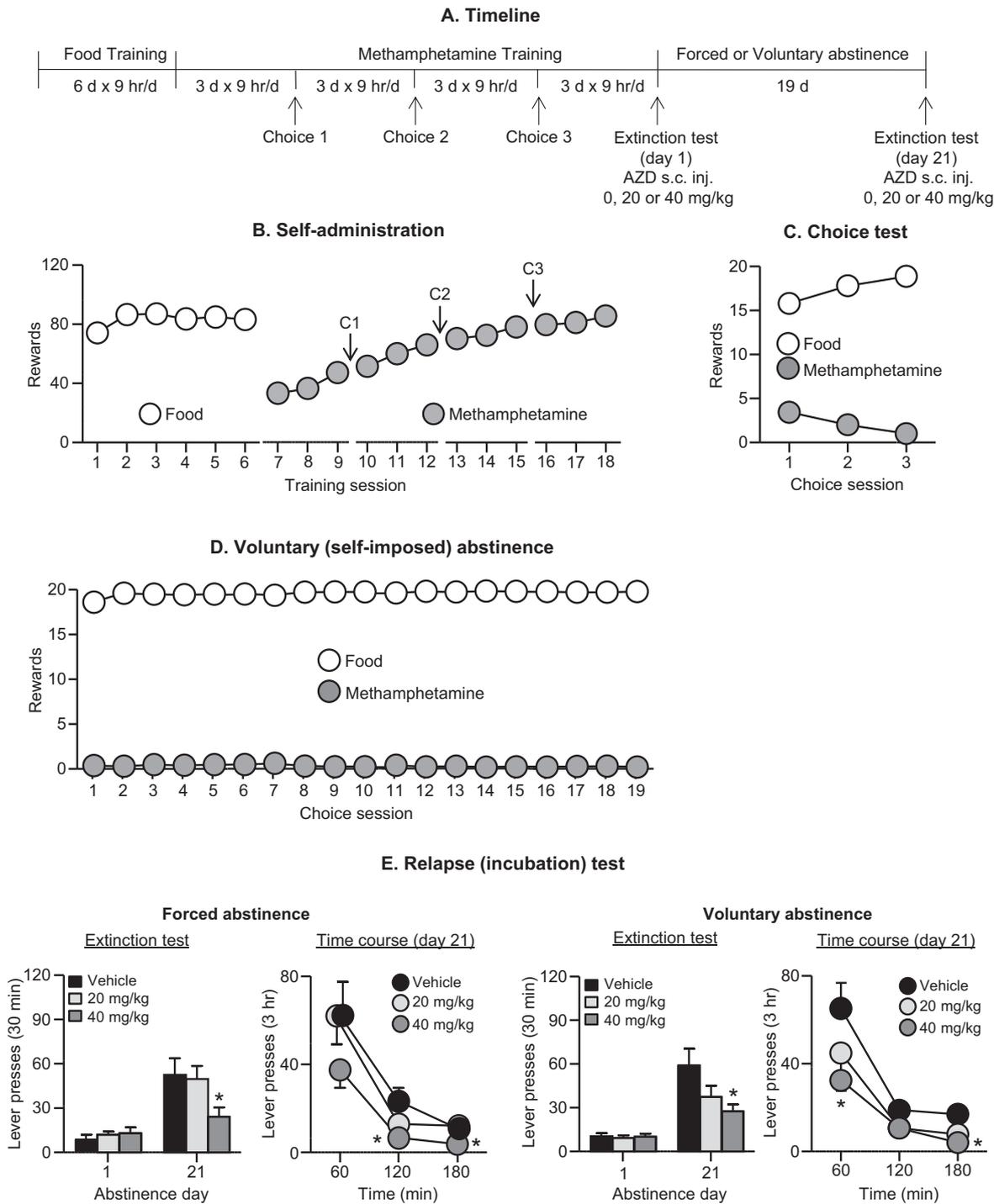
Figure 3. Effect of AZD8529 (AZD) on incubation of methamphetamine craving after forced or voluntary abstinence. **(A)** Timeline of the experiment. **(B)** Self-administration: Mean ± SEM number of food rewards or methamphetamine infusions during the 9-hour sessions (12 sessions). **(C)** Discrete choice tests: Mean ± SEM of food rewards and methamphetamine infusions earned during the three discrete choice sessions that were performed during training. **(D)** Voluntary abstinence: Mean ± SEM of food reward and methamphetamine infusions earned during the 19 discrete choice sessions. **(E)** Relapse (extinction) tests: Left side shows the data from the forced abstinence group and the right side from the voluntary abstinence group. For each condition, the left column shows active lever presses during the 30-minute extinction test on abstinence day 1 and the first 30 minutes of the 3-hour extinction test on day 21. The right column shows the time course of the 3-hour extinction test on abstinence day 21. Data are mean ± SEM of lever presses on the previously active lever during the extinction sessions. We injected vehicle or AZD8529 (20 or 40 mg/kg, subcutaneous [s.c.]) 3 hours before the test sessions. *Different from day 1 within each abstinence condition, $p < .01$; forced abstinence, $n = 8$ to 9 per dose; voluntary abstinence, $n = 14$ to 16 per dose. inj., injection.
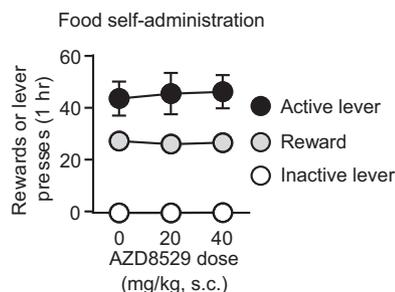
Food self-administration

These existing relapse/reinstatement models deviate from the human condition where abstinence is often voluntary due to the availability of alternative nondrug rewards (19,20). This is exemplified in the contingency management treatment procedure where the availability of nondrug rewards, given in exchange for clean urine samples, can maintain abstinence for many months (31,32,61). However, when contingency management discontinues, most addicts relapse to drug use (3,35). We propose that incubation of cue-induced drug seeking after an alternative reward choice-based voluntary abstinence in our rat model is analogous to the human condition of relapse to drug use after termination of long-term contingency management treatment. Our model also mimics to some degree relapse that occurs in more natural settings when former addicts lose important alternative nondrug rewards that maintain abstinence (a steady job, social relationships, etc).

A question that derives from our study is whether similar or different neurobiological mechanisms control incubation of methamphetamine craving after voluntary versus forced abstinence. Potential evidence for mechanistic differences is the lower magnitude of incubated cue-induced drug seeking in the voluntary abstinence condition than in the forced abstinence condition in experiment 1 (Figure 1E). However, this effect was not observed in experiment 2 under different training conditions (Figure 2E) or in experiment 3 under the same training conditions of experiment 1 (Figure 3E, vehicle groups in the voluntary and forced abstinence conditions). Additionally, AZD8529 had a similar effect on incubated cue-induced methamphetamine seeking after voluntary and forced abstinence, suggesting mechanistic similarities. Regardless, the absence of mechanistic differences in our studies using neuropharmacologic manipulations does not rule out the possibility of unique brain areas and circuits mediating incubation of drug craving after voluntary versus forced abstinence.

Finally, the results of our study may also have implications for animal models of addiction. Specifically, drug addiction is often characterized by reduced behavioral responding for nondrug rewards or diminished control over behavior by nondrug rewards (5,62,63). This aspect of human drug addiction was not observed in our study in which we used two established procedures to model addiction: extended daily access drug self-administration procedure (40,41) and long-term training procedure used to identify addicted rats based on DSM-IV criteria (42,43).

In agreement with our recent study (39) and previous observations of Ahmed *et al.* (38,64) with cocaine, the rats in our study strongly preferred the palatable food over

methamphetamine after extended access (9 hours/day) methamphetamine self-administration. We also found no evidence for a shift in food-methamphetamine choice during the extended drug self-administration training (50 sessions) in which some cocaine-trained rats develop addiction-like behavior, as assessed by progressive ratio responding, resistance to shock-induced suppression of drug-reinforced responding, and high nonreinforced responding (43,54). However, in our study, we did not explicitly monitor the emergence of these behavioral measures and our sample size was relatively small ($n = 17$). A question for future research, which will require a significantly larger sample, is whether a switch in food-drug preference would emerge in 15% to 20% of the rats who develop the addiction phenotype in the long-term training addiction model (43,54).

Effect of AZD8529 on Incubation of Methamphetamine Craving

In our initial pharmacologic characterization of incubation of methamphetamine craving after voluntary abstinence, we found that the novel mGuR2 PAM AZD8529 decreased this incubation, as well as incubation of methamphetamine craving after forced abstinence. The effect of AZD8529 on cue-induced methamphetamine seeking was only observed on abstinence day 21 but not day 1, suggesting a selective effect on incubated cue-induced drug seeking. However, this selective time-dependent effect should be interpreted with caution because of a potential floor effect due to low responding on day 1.

Our data extend the recent finding that AZD8529 decreases nicotine self-administration and cue- and drug-induced reinstatement of nicotine seeking in squirrel monkeys (46). There is also evidence that another mGluR2 PAM (BINA) decreases cocaine self-administration and cue-induced reinstatement in rats (65).

These results are in agreement with the literature on the effect of group II mGluR agonists on the behavioral effects of drugs in different animal models of addiction (66,67). Group II mGluRs comprise two Gi-coupled receptor subtypes: mGluR2 and mGluR3 (49). As mentioned in the introduction, mGluR2s are expressed primarily on presynaptic glutamate neurons and their activation leads to decreased evoked glutamate release (49,68); mGluR3s are expressed primarily on postsynaptic neurons and on glia and their physiological function is unknown (49,69). The prototype drug used to study the function of group II mGluRs is LY379268, which binds to both mGluR2 and mGluR3 (49,70). LY379268 and related compounds decreased reinstatement induced by re-exposure to discrete, discriminative, or contextual cues that were previously associated with alcohol (71), heroin (72,73), cocaine (74,75), and methamphetamine (76) self-administration. LY379268 also decreased cocaine priming-induced reinstatement in squirrel monkeys (77) and nicotine and methamphetamine self-administration in rats (78,79). We also found that systemic and central amygdala LY379268 injections decreased incubation of cocaine craving after forced abstinence (80). Based on our recent findings on the role of central amygdala in incubation of methamphetamine craving after forced abstinence (53), we speculate that mGluR2 in this brain area also plays a role in the effect of

AZD8529 on incubation of methamphetamine craving. Additionally, our data are in agreement with recent results demonstrating that inhibition of glutamate transmission in corticostriatal circuits by mGluR1 PAMs (81) or optogenetic manipulations (81–83) decreases incubation of cocaine craving.

Finally, as discussed by Justinova *et al.* (46), from a medication development perspective, AZD8529 appears to have a more favorable profile than LY379268. This is because LY379268 exhibits low bioavailability (84), tolerance can develop to its behavioral effects (78), and it activates the mGluR3 subtype whose physiological function is unknown (49). In this regard, in squirrel monkeys, Justinova *et al.* (46) did not observe any tolerance to the effects of AZD8529 on nicotine self-administration or reinstatement after repeated injections of drug doses that were well tolerated in human studies (45), making the compound a potential suitable candidate for relapse prevention.

Conclusion

We introduce a novel and reliable animal model to study incubation of drug craving and cue-induced drug seeking after voluntary abstinence, potentially mimicking the human condition of relapse after successful contingency management or other conditions that promote alternative reward choice-based abstinence. Our data further suggest that PAMs of mGluR2 should be considered for relapse prevention in psychostimulant users. Finally, our results raise two important questions for future research. First, as previous studies demonstrate that the mechanisms of opiate and psychostimulant reward and relapse/reinstatement are not the same (27,85–89), one question is whether incubation of drug craving after voluntary abstinence would also occur in rats with a history of opiate self-administration. The second question is whether similar or different mechanisms control incubation of drug craving after forced versus volitional abstinence. Our initial results with AZD8529 suggest mechanistic similarities but additional research will be required to resolve this question.

ARTICLE INFORMATION

From the Behavioral Neuroscience Research Branch (DC, MV, TZ, XL, SA, RM, NJM, GS, JMB, YS), Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, US Department of Health and Human Services, Baltimore, Maryland; Neuropsychopharmacology Laboratory (MV), Section of Pharmacology, Department Public Health and Community Medicine, University of Verona, Verona, Italy; Florey Institute of Neuroscience & Mental Health (NJM), University of Melbourne, Parkville, Victoria, Australia; and The Solomon H. Snyder Department of Neuroscience (FL), Brain Science Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

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