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

Supplemental Information

Supplemental Methods and Materials

Subjects

We used male Sprague-Dawley rats (Charles River, Raleigh, *n* = 117), weighing 300–350 g. We group-housed (2 per cage) the rats for 1-3 weeks prior to surgery and then individually-housed them after intravenous surgery. In Exp. 1 and 3, we brought the rats to the self-administration chambers on the first training day and kept them in these chambers for the duration of the experiments. In Exp. 2, we housed the rats in the animal facility and brought them to their self-administration chambers for their 3-hr daily sessions. We maintained rats on a reverse 12 hr light/dark cycle (lights off at 8 AM) with free access to standard laboratory chow and water throughout the entire experiment. Our procedures followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals (eighth edition; http://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf). We excluded 5 rats due to catheter problems and one rat due to failure to acquire methamphetamine self-administration.

Drugs

We received (+)-methamphetamine-HCl (methamphetamine) from the NIDA pharmacy and dissolved it in sterile saline. We chose a unit dose of 0.1 mg/kg for self-administration training based on our previous studies (1-3). We received AZD8529 (7-methyl-5-(3-piperazin-1-ylmethyl-[1,2,4]oxadiazol-5-yl)-2-(4-trifluoromethoxybenzyl)-2,3-dihydroisoindol-1-one) from AstraZeneca and dissolved it in sterile water (Hospira). We injected AZD8529 at doses of 20 and 40 mg/kg (free base), s.c., 3 hr prior to the extinction tests (see below). These doses are based on unpublished rat behavioral and pharmacokinetic studies of AstraZeneca and on pilot studies in which we found that AZD8529 at doses of up to 40 mg/kg had minimal effect on high rate operant responding for food pellets (data not shown).

Intravenous Surgery

We anesthetized rats with ketamine and xylazine (50 and 5 mg/kg, i.p., respectively) and inserted silastic catheters into the jugular vein as described previously (4, 5). We placed the distal end of the catheters into the jugular vein and attached the proximal end to a modified 22-gauge cannula to be placed on the back in the mid scapular region. We injected buprenorphine (0.1 mg/kg, s.c.) after surgery to relieve pain and allowed rats to recover for 7 days before palatable food and methamphetamine self-administration training. We flushed the catheters daily with 0.2 ml of sterile saline solution containing 1.0 mg of gentamicin (Butler Schein; 5 mg/mL).

Apparatus

We trained rats in self-administration chambers as described previously (5). Briefly, we equipped each chamber with a stainless steel grid floor and 2 operant panels. We equipped the left panel of the chamber with a discriminative stimulus (red light) that signaled the insertion and subsequent availability of the methamphetamine-paired active (retractable) lever. We equipped the right panel of the chamber with a discriminative stimulus (white house light) that signaled the insertion and subsequent availability of the food-paired active (retractable) lever. We equipped the right wall with an inactive (stationary) lever that had no reinforced consequences. We placed a bottle of water and a food hopper on the side of the chamber's transparent polycarbonate door, respectively.

Methamphetamine and Food Self-Administration Training

Methamphetamine self-administration

The procedure is similar to the one described elsewhere (1, 3, 5). Briefly, we trained rats to self-administer methamphetamine during three- or nine-1-hr daily sessions (see specific experiments below) that were separated by a 10-min off period, under a fixed-ratio-1 (FR1) 20-sec timeout reinforcement schedule; drug infusions were paired with the 20-sec discrete white light cue. The sessions began with the presentation of the red light for 10 sec followed by the insertion of the methamphetamine-paired active lever; the red light remained on for the duration of the session and served as a discriminative stimulus for methamphetamine availability. At the end of each 1-hr session, the red light was turned off,

and the active lever was retracted. The rats self-administered the drug at a dose of 0.1 mg/kg/infusion over 3.5 sec (0.1 ml/infusion). To prevent overdose, we limited the number of infusions to 15 per hr.

Food pellets self-administration

Our palatable food training procedure was similar to that used for methamphetamine with the following exceptions. First, the lever presses under the FR1, 20-sec timeout reinforcement schedule led to the delivery of five 45-mg 'preferred' or palatable food pellets (TestDiet, Catalogue # 1811155, 12.7% fat, 66.7% carbohydrate, and 20.6% protein); pellet deliveries were paired with the 20-sec discrete tone cue. [Note: we gave the rats 5 pellets per reward delivery in order to approximate the response rate of food- and methamphetamine-reinforced responding (1)]. Second, prior to the first 1-2 formal operant training sessions, we gave the rats 1-hr magazine training sessions during which 5 pellets were delivered non-contingently every 5 min. The sessions began with the presentation of the white house light followed 10 sec later by the insertion of the food-paired active lever; the white house light remained on for the duration of the session and served as a discriminative stimulus for the palatable food. At the end of the session the white light was turned off and the active lever was retracted.

We have used the 'preferred' TestDiet pellet type in recent food reinstatement studies (6-8) and the present study, because in food preference tests, rats prefer this type of pellet over other pellet types with different compositions of fat and carbohydrate, and different flavors (9). Furthermore, in our recent parametric study, we have shown that rats strongly prefer these food pellets over intravenous methamphetamine (5).

Discrete-trials choice procedure

We conducted the discrete choice sessions using the same parameters (dose of methamphetamine, number of palatable food pellets per reward, stimuli associated with the two retractable levers) that we used during the training phase. We allowed rats to choose between the methamphetamine- and a palatable food-paired lever in a discrete-trials choice procedure, as previously described (10, 11). We divided each 200 min choice sessions into 20 discrete trials that were separated by 10 min. We chose this interval because it is longer than the inter-infusion interval under our training conditions (about 5-7 min) and thus, prevents a potential confound of drug 'satiety', which can bias choice toward the food. Drug 'satiety' periods between consecutive drug injections (analogous to food satiety periods between

consecutive food deliveries) are controlled by fluctuations in brain drug levels and are highly correlated with dopamine levels in nucleus accumbens (12, 13). According to Tsibulsky and Norman (12), the inter-infusion interval during psychostimulant self-administration is determined by within-session drug craving that is maximal when drug accumulated from previous injections is metabolized below a "satiety threshold." We also chose the 10 min inter-choice interval over shorter time periods in order to reduce the direct anorexigenic effect of methamphetamine accumulation on food intake (14) and consequently food choice.

The 10 min inter-trial interval in the discrete choice sessions results in intermittent access to food and methamphetamine and this parameter could potentialy change drug self-administration patterns and drug seeking during abstinence. Indeed, it has recently been shown that an intermittent access to cocaine self-administration results in sensitization of both cocaine reinforcing effects and dopamine signaling (15, 16). However, these studies differ from the present study in several aspects, including drug exposure (12 five-min trials separated by 25-minute timeout periods), the self-administration drug (cocaine) and lack of an alternative reward. However, as we observed persistent food choice in the different phases of our experiment, it is unlikely that the particular parameters we have used in the choice session had an effect on either methamphetamine self-administration or extinction responding during the test sessions on abstinence days 1 and 21.

Each trial began with the presentation of both discriminative stimuli previously associated with palatable food or methamphetamine followed 10 sec later by the insertion of both palatable food- and methamphetamine-paired levers. Rats then had to select one of two levers. If rats responded within 8 min, they received the reward corresponding with the selected lever (in the present set of experiments the rats pressed either the food- or the methampehtamine-paired lever in all 20 discrete choice trials and ate the palatable food pellets earned). Reward delivery was signaled by the methamphetamine- or food-associated cue (white cue light or tone, respectively), the retraction of both levers, and the turning off of both food- and methamphetamine discriminative cues. If a rat failed to respond on either active lever within 8 min, both levers were retracted and their related discriminative cues were extinguished with no reward delivery (5). We introduced choice sessions during the training phase in order to assess whether the choice behavior changes over time during this phase.

Abstinence Phase

After the completion of the training phase, we divided the rats into two conditions: forced abstinence and voluntary abstinence. We gave rats in the forced-abstinence group free access to palatable food pellets in a ceramic bowl (100 pellets = 4.5 g; 19 days) in their operant chamber (Exp. 1 and 3) or in their home cage (Exp. 2). We provided non-operant access to the food in the forced-abstinence condition because we wanted to equate the amount of food consumed during the abstinence phase between the forced and voluntary abstinence conditions while keeping the other experimental parameters of the forced abstinence groups similar to the ones used in previous studies on incubation of drug craving after forced abstinence. We allowed the voluntary abstinence rats to choose for 19 sessions between the methamphetamine- and a palatable food-paired lever (delivering 5 pellets) during 20 discrete-choice trials, as described above.

Extinction Tests

The extinction tests in the presence of the methamphetamine-associated cues consisted of a single 30-min session on abstinence days 1 and 21 in Exp. 1-2, and a 3-hr session on day 21 in Exp. 3. The sessions began with the presentation of the red discriminative cue light followed 10 sec later by the insertion of the methamphetamine-paired active lever; the red light remained on for the duration of the session. Active lever presses during testing, the operational measure of cue-induced drug seeking in incubation of craving studies (17, 18), resulted in contingent presentations of the light cue, previously paired with drug infusions, but not methamphetamine (1, 2, 19).

Statistical Analyses

We analyzed the data with the statistical program SPSS and followed significant effects ($p < 0.05$) with SPSS post-hoc contrasts within the repeated measures ANOVA module. For the training phase, we analyzed the data separately for food rewards and methamphetamine infusions, using the within-subjects factor of Session. For the choice tests, we normalized at 0 the indifference level between palatable food pellets and methamphetamine (preference score) using the following formula: 1 - (% drug choices/50%) (11) and analyzed the data with repeated-measures ANOVA, using the within-subjects factor Choice

session. For the relapse tests in Exp. 1-2, we analyzed non-reinforced active lever-presses in the extinction sessions, using the between-subjects factor of Abstinence Condition (forced, voluntary) and the within-subjects factor of Abstinence Day (1, 21). For the relapse tests in Exp. 3, we analyzed the active lever-press data using the between-subjects factors of Abstinence Condition (forced, voluntary), AZD8529 Dose (0, 20, 40 mg/kg), and the within-subjects factor of Abstinence Day (1, 21). We also analyzed the 3-hr time course of active lever-presses during the day 21 extinction test, using the between-subjects factors of Abstinence Condition and AZD8529 Dose, and the within-subjects factor of Session Hour (1, 2, 3). In all analyses of the relapse tests, we used inactive lever-presses as a covariate in order to statistically control for the effect of abstinence period on non-specific (training independent) lever presses during testing.

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