

Elier et al – mGlu₅ receptors and relapse

A medium throughput rodent model of relapse from addiction with behavioral and pharmacological specificity

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Non-standard Abbreviations

ABT-431- diacetyl prodrug form of (5 a R, 11 b S)-4,5,5a,6,7,11b-hexahydro-2 propyl-3-thia-5-azacyclopent-1-ena[c]-phenanthrene-9,10-diol (A-86929)

FR1 – fixed-ratio 1

LY379268 - (1R,4R,5S,6R)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid

MPEP - 2-Methyl-6-(phenylethynyl)pyridine

MTEP - 3-((2-Methyl-4-thiazolyl)ethynyl)pyridine

ABSTRACT

One of most formidable problems in the treatment of addiction is the high rate of relapse. The discovery of medicines to help mitigate relapse are aided by animal models that currently involve weeks of training and require surgical preparations and drug delivery devices. The present set of experiments was initiated to investigate a rapid 8-day screening method that utilizes food instead of intravenous drug administration. Male Sprague-Dawley rats were trained in a reinstatement paradigm in which every lever press produced a 45mg food pellet concurrently paired with a light and tone. Behavior was subsequently extinguished with lever responses producing neither food nor food-associated stimuli. Reinstatement of responding was evaluated under conditions in which the first three responses of every 5 min time bin produced a food pellet along with food-associated stimuli. The mGlu₅ receptor antagonists MPEP and MTEP produced a significant reduction in reinstatement while failing to alter responding where every response produced food. The cannabinoid CB₁ receptor antagonist rimonabant and the mGlu_{2/3} receptor agonist LY379268 also selectively reduced reinstatement. Other compounds including clozapine, *d*-amphetamine, chlordiazepoxide, ABT-431, naltrexone and citalopram were without effect. The results suggest that relapse-like behavioral effects can be extended to non-pharmacological reinforcers. Drug effects demonstrated both behavioral and pharmacological specificity. The present experimental design thus allows for efficient and rapid assessment of the effects of drugs that might be useful in the treatment of addiction-associated relapse.

INTRODUCTION

One of most formidable problems in the treatment of addiction is the high rate of relapse following periods of prolonged abstinence (Hunt et al. 1971; O'Brien 1997). In humans, initiation of drug-taking behavior following abstinence is typically preceded by craving for the abused drug. Motivation to re-engage patterns of drug-seeking and drug-taking can be produced by the drug itself or by the presentation of stimuli associated with drug taking behavior (Wikler, 1973; Wikler and Pescor, 1967).

Initial work on the creation of an animal model of human drug relapse began over three decades ago with the seminal work of Stretch and colleagues (1971) and Davis and Smith (1976). Little has changed since the emergence of the paradigm with the basic methodology remaining essentially the same. Animals are first trained to produce responses by reinforcing those responses by drug infusion. Following acquisition of drug-taking, the behavior is extinguished by removal of the drug and drug-associated stimuli. Drug-seeking behavior is then reinstated by the presentation of a reinstatement trigger that can include exposure to the drug itself or drug associated stimuli (Jaffe et al. 1989; Ludwig et al. 1974; O'Brien et al. 1992) or the presence of a stressor (Brown et al. 1995; Sinha, 2001). Each of these factors has been shown to produce self-reports of craving and relapse in abstinent humans (Childress et al., 1999; Robbins et al., 1999; Walsh et al., 2000; Wasserman et al., 1998).

Animal models of relapse generally use intravenous drugs as reinforcers requiring surgical preparations and drug delivery devices. In addition, these relapse models require long training periods before novel potential anti-craving compounds can be evaluated. For example, Kim et al. (2015) used intravenous cocaine administration requiring surgical implantation of indwelling intravenous catheters followed by recovery time, 12 days of cocaine self-administration training, followed by 9 days of extinction training. In the present set of experiments, we utilize food as a reinforcer in a paradigm that allows anti-relapse drug testing in 8 days. The idea behind this method is the overlap in functional behavioral and biological substrates for reinforced behaviors that transcend the specific reinforcing stimulus (drug or food).

Commonalities in the functional control of behavior by diverse reinforcers have been demonstrated (Kelleher and Morse, 1968; Barrett, 1987; Witkin and Katz, 1990). It is increasingly recognized that the behavioral bases and biological substrates underlying addictions of drug- and non-drug stimuli share common ground (c.f., Chamberlain et al., 2016; Chen et al., 2017; Rogers, 2017; Volkow et al., 2012, 2017). Nonetheless, the generality of relapse phenomenon to non-drug reinforcers has undergone little experimental scrutiny. Studies have shown that stimuli associated with either food reward or pharmacological reinforcers can produce motivation to seek out and to crave these reinforcers (Brody et al., 2002; Childress et al., 1999). Such a motivational state is what is thought to produce relapse not only to drug-taking behavior, but as Berthoud (2004) suggests, relapse to feeding as well. For example, the anxiogenic drug, yohimbine,

can reinstate behavior following a period of extinction in animals previously trained to self-administer food just as it has after drug reinforcers (Ghitza et al., 2006).

The over-arching goal of the present study was to evaluate the utility of a new animal model of food reinstatement developed from modifications of the classic drug-reinstatement paradigms. We first established the reliability of generating reinstatement of extinguished behavior after only 8 days. Next, we determined the relative efficacy of different conditions to induce reinstatement of behavior. Subsequently, several drugs were tested under these conditions to assess their ability to dampen reinstated behavior. The drugs selected were based upon those used in patients and those suggested to be anti-craving drugs in the preclinical scientific literature. These drug effects were compared to effects of other compounds that are generally not thought of as anti-craving medications. Thus, these pharmacological studies begin to ascertain 1) whether the 8-day model has predictive validity for detecting anti-relapse drugs and rejecting drugs that are not applicable for anti-relapse therapeutics, and 2) whether a food-based reinstatement model detects comparable pharmacological mechanisms to those detected with drug reinforcer-based models. In addition to beginning to define pharmacological specificity in this model, we utilized control behaviors (non-reinstated behaviors) to determine if the drug effects on reinstatement are behaviorally specific. It is argued that drugs that dampen relapse behaviors at doses that do not affect the non-relapse behaviors should be considered as specific pharmacological mechanisms for relapse prevention.

Drugs that have some validation as anti-craving medications selected for investigation are briefly outlined here. The CB₁ receptor antagonist rimonabant, that has been used as an anti-obesity drug (Rubio et al., 2007) was predicted to reduce craving (Kirkham, 2008) and has demonstrated reductions in hunger in humans (Koch et al., 2017) and craving in animal models of reinstatement (Cohen et al., 2005; De Vries et al., 2001; Piomelli, 2001; Schindler et al., 2016; Ward et al., 2009). In addition to opioid abuse disorders, naltrexone is used in the treatment of alcohol addiction where it reduces craving in humans (Hendershot et al., 2017) and attenuates reinstatement to some drugs of abuse in animal models (Anggadiredja et al., 2004; Ciccocioppo et al., 2003; Liu and Weiss, 2002).

Dampening of glutamate neurotransmission by mGlu₅ receptor antagonism has been suggested as an anti-relapse mechanism from reinstatement studies in rodents (Bäckström et al., 2004; Bespalov et al., 2005; Kim et al., 2015; Knackstedt and Schwendt, 2016; Pomierny-Chamiolo et al., 2017a; Richard, 2019; Tessari et al., 2004). Likewise, reducing glutamate outflow by mGlu_{2/3} receptor agonists has also been shown to be an effective method of reducing reinstated behaviors in rodent models (Baptista et al., 2004; Pomierny-Chamiolo et al., 2017b) or in primates (Justinova et al., 2016). Drugs that are not considered to have anti-craving properties were studied for comparison: the antipsychotic drug clozapine, the psychostimulant, *d*-amphetamine, the anxiolytic drug chlordiazepoxide, the antidepressant citalopram, and the dopamine receptor agonist ABT-

431. Although some justification is provided for the compounds used for assay validation in the present study, it is recognized that until there are medicines proven for their efficacy against drug abuse relapse and/or food-related behaviors, these tools remain imperfect. The validation of predictive animal models depends upon existing and emerging data gained from controlled studies from both the preclinical and clinical laboratories that reciprocally inform one another (Willner, 1984).

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MATERIALS AND METHODS

Subjects. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) weighing 275-305 g upon arrival were used for all phases of this study. A total of 475 rats were used in these experiments. These animals were housed individually to allow for food restriction to produce animals weighing ~85% of their free feeding weight. All animals were fed daily. While food was restricted, all animals had free access to water. All animals were placed on a normal light/dark cycle with lights on at 6:00 am and off at 6:00 pm with experiments conducted between 9:00 am and 4:00 pm. Animals were removed from the vivarium in their home cages and transported to the testing room. All experiments were conducted according to the Guidelines for Care and Use of Laboratory Animals under protocols approved by a local animal care and use committee and under monitoring by veterinary staff.

Apparatus. Animals were tested in 14 standard rat operant chambers (Med Associates, Inc, St. Albans, VT, USA) equipped with 2 levers on a single wall with a pellet delivery assembly with the food trough located between the levers (see Alt et al., 2005). Each chamber was also equipped with a house light, as well as white cue lights above each lever and an audio tone generator (sonalert @4500 Hz and 65dB). The grid floor of the chamber enabled delivery of scrambled electric shock.

Drugs and Food Pellets. A number of psychoactive drugs were tested in this study in order to evaluate the dynamics of the new reinstatement paradigm. These drugs include the antipsychotic clozapine, the benzodiazepine anxiolytic chlordiazepoxide, the dopamine D₁ receptor agonist ABT-431, the dopamine releaser *d*-amphetamine, the pan opioid receptor antagonist naltrexone, and the selective serotonin reuptake inhibitor citalopram which were purchased from Sigma Chemical Company (St. Louis, MO). We also studied, the cannabinoid CB₁ receptor antagonist SR141716A (rimonabant), the mGlu_{2/3} receptor agonist LY379268, and the mGlu₅ receptor antagonists MPEP and MTEP which were synthesized at the Lilly Research Labs. All agents were administered via intraperitoneal (i.p.) injection 30 min before the start of the test session in an injection volume of 1.0 ml/kg. Clozapine, chlordiazepoxide, ABT-431, LY379268, MPEP, MTEP, naltrexone and citalopram were dissolved in sterile water, while rimonabant was dissolved in a suspension of 1% CMC, 0.5% SLS, 0.085% providone, and 0.05% antifoam.

The food pellets used in this study were 45mg dustless precision pellets (#F0021) manufactured by Bio-Serv products (Frenchtown, NJ, USA).

Reinstatement Paradigm.

Response Training: Upon arrival, animals were allowed at least three days to habituate to their new environment before testing was begun. Following the habituation period, animals were tested once daily in a 30 min food self-administration session. Three 45mg food pellets were placed in the food trough prior to the first test session to help animals associate the trough with food delivery. During all training sessions, responses on the right lever delivered one 45mg food pellet and produced the concurrent presentation of an audible tone and house light illumination both lasting 2 sec. Responses on the right lever were reinforced under a fixed ratio (FR) 1 schedule of reinforcement whereby each lever press produced food. Responding on the left lever produced no reinforcer or associated stimuli. Each session ended when either 50 pellets had been delivered or 30 min elapsed, whichever occurred first. Training continued until all animals earned all 50 food pellets within the 30 min session for two consecutive sessions. Any animals that failed to meet this criterion within 7 sessions were excluded from the experiment. This value by experiment was relatively low (2.1%). This phase of the experiment lasted 3 days.

Extinction: The extinction phase began immediately after the subjects had successfully acquired the response training criterion noted above and lasted for 4 consecutive experimental sessions. During extinction, animals were tested daily for a 30 min experimental session during which responses on the right lever did not produce food pellets or the reinforcer-associated stimuli (i.e. tone and house light). As during acquisition, responding on the left lever produced no reinforcer or food-associated stimuli. Animals continued responding under extinction until their responses had stabilized ($\pm 20\%$) for three consecutive days.

Reinstatement: Following extinction, food reinstatement was evaluated. In this portion of the experiment, subjects were placed in the operant chamber for a 30 min experimental session separated into 5 min time bins. During reinstatement, only the first three right lever responses of each 5 min period were reinforced by a 45mg pellet and the presentation of the food-associated stimuli. Subsequent responses on the right lever produced only the tone and house light illumination. As before, responses on the left lever were recorded but not reinforced. Reinstatement sessions ended after 100 right lever responses or when 30 min had elapsed, whichever occurred first.

Second Reinstatement: In order to best utilize experimental animals, all rats were studied under a second reinstatement test. This phase began with the reacquisition of the food self-administration behavior on the day following the initial reinstatement session. During reacquisition, animals were tested once daily using the same parameters as the training sessions described above. Animals were tested over 3 to 4 sessions to ensure that all subjects responded to criterion, earning the maximum 50 reinforcers for 3 consecutive sessions. After reacquisition of responding, the rats entered a second set of extinction sessions which continued until responding had stabilized. Following extinction, animals underwent a second reinstatement session identical to the one

described above. This allowed for the testing of a second drug or drug vehicle using the same animals. No additional tests were conducted on these rats. Thus, in most, but not all experiments, the same rats used for one drug or vehicle test, were used again in one additional experiment. A total of 5-9 rats were used for each experimental data point.

Comparison of Reinstatement Stimuli. A host of stimuli were evaluated to establish the comparative efficacy of these stimuli to reinstate responding after extinction. Following extinction, stimuli that might produce relapse to food-seeking were evaluated the next day (day 8). The following stimuli were studied: response-produced presentations (FR1) of a) food-associated stimuli (tone + light), b) food alone, c) food plus food-associated stimuli, d) food plus food-associated stimuli for the first three responses only, e) foot-shock (0.1 mA, for 100 msec) in the absence of food or food-associated stimuli, and response-independent food delivery when food was delivered along with food-associated stimuli every 30 sec. Experimental sessions continued for 60 min or 100 presentations of stimuli, whichever occurred first.

Fixed Ratio (Maintenance) Paradigm. In these experiments, rats were maintained under a schedule of response-produced food delivery. Responses were not extinguished or reinstated as in the reinstatement conditions described above. Drug effects under this maintenance condition could then be compared to drug effects under the reinstatement condition to assess the behavioral specificity of drug effects on reinstated behavior.

Food Training: All animals were allowed at least three days to habituate to their new environment after arrival to the vivarium before testing was begun. Animals were then trained under the FR1 schedule of food delivery as described above. Training continued until all animals earned all 100 possible reinforcers within the 30 min test session for two consecutive sessions. Any animals that failed to meet this criterion within 7 sessions were excluded from the experiment. This value by experiment was relatively low (1.3%).

Evaluation of Drug Effects: After training, responding was evaluated in these animals following drug administration. Subjects were placed in the operant chamber for 30 min and each right lever response was reinforced by a 45mg pellet and the associated stimuli just as in the training sessions above. As before, responses on the left lever were recorded but not reinforced. Each session ended after 100 right lever responses had been made or when 30 min had elapsed, whichever occurred first.

Data Analysis. Data were collected for the mean number of responses. Entry into the food trough was not recorded. A one-way analysis of variance (ANOVA) was performed on dose-effect data for each compound. Two-way ANOVA was used to evaluate data over experimental sessions (treatment x experimental session). Significant overall ANOVA values were subsequently analyzed by Dunnett's post-hoc test.

RESULTS

Reinstatement of Food-Maintained Responding by Food Presentation and/or by Discriminative Stimuli.

The ability of different stimuli to reinstate responding after extinction was examined and the data are shown in **figure 1**. Comparing all reinstatement conditions to one another resulted in a significant effect of treatment condition [$F(5,47) = 16.4, p < 0.0001$]. All stimuli investigated produced reinstatement of responding that was significantly greater than responding on the last day of extinction. The greatest reinstatement was produced by response-produced food and response-produced food with concurrently-delivered food-associated stimuli. All other inducers of reinstatement produced lower and roughly equivalent levels of responding. The reinstatement condition of response-produced food + food-associated stimuli for the first 3 responses was then utilized to evaluate drug effects as reported below.

Figure 2 illustrates responding under the food self-administration paradigm during acquisition, extinction, and reinstatement. All animals successfully acquired the lever press response (right lever) and reached criterion of 50 food deliveries within the 30 min experimental session by day 2 of training. During acquisition, there were no statistical differences across the three groups of rats [$F(2,57) = 0.63, p > 0.05$], across experimental sessions [$F(2,57) = 0.16, p > 0.05$], and no group x session interaction [$F(4,57) = 0.36, p > 0.05$].

During extinction, responses on the right lever significantly declined relative to responding during the acquisition period with significant differences compared to the last day of acquisition by extinction day 2. Two-way ANOVA confirmed a significant effect of experimental session on behavior [$F(3,76) = 9.8, p < 0.0001$]. There were no statistically-significant differences in extinction across groups [$F(2,76) = 0.32, p > 0.05$] and no session x group interaction [$F(6,76) = 0.98, p > 0.05$].

Three stimulus configurations were delivered on the day following extinction (day 8). There was a significant effect of treatment group on reinstated behavior [$F(2,21) = 8.7, p < 0.001$]. In this experiment as with the data in figure 1, food-paired stimuli alone were sufficient to significantly reinstate extinguished responding (**Fig. 2**). Response-produced food delivery alone (first 3 responses of each 5 min time bin) or response-produced food in the presence of food-associated stimuli produced large increases in responses post extinction. (**Fig. 2**).

Reinstatement of Food-Maintained Responding following Drug Administration. **Table 1** shows the effect of multiple pharmacological agents on the reinstatement of food self-administration. Only rimonabant, LY379268, MPEP, and MTEP were effective in altering reinstated responding. Clozapine, *d*-amphetamine, chlordiazepoxide, ABT-431,

naltrexone, and citalopram all failed to affect the reinstatement of food self-administration as evaluated by this paradigm.

Along with their effects on reinstatement, each of the compounds was evaluated under a FR1 schedule (response maintenance condition) to determine the behavioral specificity of any effects observed on reinstatement. These data are summarized in **Table 1**. Under these conditions, only LY379268 and MTEP altered responding under an FR1 schedule of reinforcement (response maintenance) with all other tested compounds failing to affect mean responding.

Effect of the mGlu₅ Receptor Antagonist MTEP on Reinstatement of Food-Maintained Responding: **Figure 3** (top panel, black bars) illustrates the effect of MTEP (3.0 -10.0 mg/kg) on responding following reinstatement. MTEP significantly suppressed response reinstatement [$F(3, 26) = 16.64, p < .01$] with 5.6 and 10.0 mg/kg significantly separating from vehicle control.

MTEP was also studied in rats responding under FR1 schedules of food delivery (response maintenance condition). MTEP did not significantly affect responding under the FR1 schedule (**Fig. 3**, top panel, checked bars) [$F(3, 26) = 2.03, p > 0.05$].

Effect of the mGlu₅ Receptor Antagonist MPEP on Reinstatement of Food-Maintained Responding: Effects of MPEP (3.0 - 10.0 mg/kg) on responding following reinstatement is depicted **figure 3** (bottom panel, black bars). As with MTEP, the 5.6 and 10.0 mg/kg doses suppressed reinstatement with the 3.0 mg/kg failing to effect responding when compared to vehicle [$F(3, 35) = 10.99, p < .01$].

MPEP did not decrease responding under the response maintenance FR1 schedule [$F(3, 35) = 1.39, p > .05$] even at doses that suppressed response reinstatement (**Fig. 3**, bottom panel, checkered bars).

Effect of the mGlu_{2/3} Receptor Agonist LY379268 on Reinstatement of Food-Maintained Responding: LY379268 significantly reduced responding under the reinstatement condition [$F(3, 34) = 37.2, p < .01$]. Post-hoc testing indicated that the 3.0 and 5.6 mg/kg doses of LY379268 produced significant ($p < 0.01$) suppression of responding while the 1.0 mg/kg dose failed to alter lever pressing (**Fig. 4**, top panel, black bars).

LY379268 also reduced responding under the response maintenance FR1 schedule of food delivery [$F(3, 17) = 27.7, p < 0.01$]. The dose of 5.6 mg/kg produced a significant ($p < 0.01$) reduction in responding as compared to vehicle while the lower doses of 1.0 and 3.0 mg/kg had no significant effect (**Fig. 4**, top panel, checkered bars).

Effect of the CB₁ Receptor Antagonist Rimonabant on Reinstatement of Food-Maintained Responding: Rimonabant (10.0 mg/kg) significantly ($p < 0.01$) reduced

reinstated responding [$F(1, 14) = 4.73, p < 0.05$] (**Fig. 4**, bottom panel, black bars). Rimonabant failed to produce any effect on the maintenance of food-maintained responding under the FR1 schedule of response maintenance [$F(1, 14) = 0.74, p > 0.05$] (**Fig. 4**, bottom panel, checkered bars).

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DISCUSSION

Commonalities in behaviors, controlling variables, and biological substrates exist across drug- and non-drug reinforcers that initiate and maintain addictions (c.f., Chamberlain et al., 2016; Chen et al., 2017; Rogers, 2017; Volkow et al., 2012, 2017). For example, presentation of either morphine- or food-associated stimuli increased expression of NGFI-B in the medial and lateral portions of the prefrontal cortex (Kelley et al., 2005). Building upon the idea of reinforcer homology, we created a relatively rapid and non-surgical method for evaluating drugs for their potential to treat relapse in addiction states using food reinforcement. In contrast to the many weeks generally used in reinstatement models, we present data on a new method that generates assay results in 8 days.

We first showed that reinstatement of behavior could be induced by a host of stimuli. All experimental conditions investigated produced reinstatement of responding that was significantly greater than extinction response levels. These included stimuli associated with the reinforcer of food delivery, the reinforcer itself, and the stressor of foot shock. Thus, stimuli known to induce reinstatement of drug-taking (Shaham et al., 2003) were effective in reinstating food-seeking behavior in the current model as well.

A start to the pharmacological validation of the model was also presented here. Drugs used for treatment of addiction craving in patients, naltrexone and rimonabant (see Introduction for references) were studied. Rimonabant selectivity reduced relapse to food-seeking behavior, whereas naltrexone did not. The lack of activity of naltrexone in this model might point to the lack of firm data on the potential use of naltrexone in reducing hunger for food and food craving. However, the comparative data on naltrexone in non-food-based relapse models is also not extensive and there are reports of lack of efficacy (c.f., Comer et al., 1993). Naltrexone is also not markedly effective alone in treating obesity (Narayanaswami and Dvoskin, 2017). In contrast, the efficacy of rimonabant is congruent with findings with food addictions as well as drug reinforcers (see Introduction). Further pharmacological validation of the current model with positive controls will require clinical efficacy of new agents and the firm establishment of efficacy of existing agents. This is a situation faced in the validation of all animal models as prefaced in the introduction.

Although drugs were shown here to attenuate relapse to food-seeking behaviors, the mechanisms by which they produced this effect is likely complex. For example, suppression of reinstatement could be generated by the induction of a state distinct from the non-drug food-taking state (state-dependency, e.g., Self and Choi, 2004). Arguments against state-dependency can be made (e.g., not all drugs suppressed reinstatement and FR1 responding was differentially affected by some drugs that reinstated responses). Multiple other mechanisms should also be scrutinized and experimentally tested. For example, drug effects on appetite might have influenced the reinstatement process. If

that were the case, one would predict *d*-amphetamine and rimonabant, appetite suppressants, to decrease responding. However, increases in FR1 responding were reported with *d*-amphetamine and no effect was shown with rimonabant on FR1 responding. Although, it is known that drug effects on behavior do not always follow motivational theory (c.f., Kelleher and Morse, 1968; Witkin and Katz, 1990), it is important to scrutinize all possibilities when exploring new models.

In addition to rimonabant, two glutamatergic mechanisms that have been implicated in relapse (see Introduction for references), mGlu_{2/3} receptors and mGlu₅ receptors were also investigated. These receptor mechanisms were explored using specific pharmacological ligands, LY379268 (mGlu_{2/3} receptor agonist) MPEP (mGlu₅ receptor antagonist), and MTEP (mGlu₅ receptor antagonist). All three compounds selectively reduced reinstatement of food-seeking behavior. Some drugs not typically associated with relapse prevention were also studied. Clozapine, *d*-amphetamine, chlordiazepoxide, ABT-431, and citalopram were not effective in reducing relapse to food-seeking. Other compounds that have shown efficacy in attenuating reinstatement of behavior by drugs, such as memantine (Popik et al., 2006), were not studied here but could be employed in future studies for model validation. Additional studies are also needed with drugs with efficacy in patients (e.g., lorcaserin) (Narayanaswami and Dwoskin, 2017) and those that are not.

In addition to pharmacological specificity, we also began to explore the behavioral specificity of drug action. Theoretically, a drug that reduces reinstatement should do so without affecting ancillary behaviors. For example, if a drug reduced reinstatement of food-seeking but also decreased the ability to normally find and eat food, this would not be a valuable medicine. We found that for the drugs that reduced reinstatement in the present study (rimonabant, MTEP, MPEP, and LY379268), there was a separation in doses that decreased reinstated behavior and doses that decreased normal ongoing food-maintained behavior. Work will need to be done to further address the question of behavioral specificity. In the present study, the baseline maintenance behavior (FR1) was not identical in all ways to the reinstated behavior – different amounts of behavior generated and different schedules of food delivery.

The data on the glutamatergic ligands also adds to the experimental literature suggesting value for glutamatergic mechanisms as potential anti-addiction agents based upon neuroanatomical (Childress et al., 1999; Garavan et al., 2000; Grant et al., 1996; Kilts et al., 2001; Maas et al., 1998; Wang et al., 1999; Wexler et al., 2001; Tzschentke, 2001; Tzschentke and Schmidt, 2000) and functional (Cornish and Kalivas, 2000; McFarland and Kalivas, 2001; Meil and See, 1997; Richard, 2019) outcomes as well as data from reinstatement models with mGlu₅ receptor antagonists and mGlu_{2/3} receptor agonists (see Introduction). We have previously shown that an mGlu₅ receptor antagonist reduces relapse to food-seeking in wild-type mice but not in mGlu₅ ^{-/-} mice (Elier et al.,

2011). The utility of this mechanism in food craving might now be added to the list of potential therapeutic applications.

We conclude that the 8-day assay method presented here might provide a method for the rapid detection of new pharmacological approaches to the treatment of relapse associated with addictions. Although the model uses food as a reinforcer, the pharmacological data at present point to the possibility of generality to addictive behaviors as a whole. The evidence suggests this possibility since drugs (with the possible exception of naltrexone) that were active in preclinical relapse models using drugs as reinforcers were active in the food-based model studied here. It should be noted that unlike chronic addiction phenomenon for drugs (e.g., Staples et al., 2015) or food (Butler and Eckel, 2018; Lee and Dixon, 2017) that generally develop over long periods of time and involve neural remodeling, the present model might not recapitulate these neuroplasticity dynamics.

It is well-known that despite commonalities in controlling variables and neurobiological substrates across reinforcers as discussed here, different reinforcers also control behavior in distinct ways (c.f., Banks, 2017; Martin-Fardon and Weiss, 2017). Thus, the likelihood of this model being able to provide precise prediction of drug effects across all reinforcer domains should also not be counted on. Nonetheless, the ability to generalize to multiple relapsing phenomenon is important: there are many stimuli that guide addictive behaviors (drugs, gambling, shopping, internet use, etc.) and all have been shown to have deleterious impacts on lives. As with all preclinical models, ultimate validation must come from data on the efficacy of compounds in patients. The medical need for improved medicines in this domain is high from both a health (e.g., Brady et al., 2016) and economic (e.g., Winkler et al., 2017) perspective.

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TABLE LEGEND

Table 1. Summary of the effects of various drugs on reinstatement and maintenance of food-maintained responding.

Mechanism	Compound	Dose (mg/kg)	Reinstatement	FR1
Antipsychotic/D ₂ receptor antagonist	chlorpromazine	5.6	NS	NS
Anxiolytic/GABA _A receptor potentiator	chlordiazepoxide	20	NS	NS
D ₁ receptor agonist	ABT-431	1	NS	Not tested
Indirect dopamine agonist	<i>d</i> -amphetamine	3	NS	↑
CB ₁ receptor antagonist	rimonabant	10	↓	NS
mGlu _{2/3} receptor agonist	LY379268	1	NS	NS
		3	↓	NS
		5.6	↓	↓
mGlu ₅ receptor antagonist	MPEP	3	NS	NS
		5.6	↓	NS
		10	↓	NS
mGlu ₅ receptor antagonist	MTEP	3	NS	NS
		5.6	↓	NS
		10	↓	NS
Opioid receptor antagonist	naltrexone	10	NS	NS
Antidepressant /Selective serotonin reuptake inhibitor	citalopram	3	NS	Not tested

Arrows indicate significant reductions or increases in responding when compared to vehicle. NS: not significantly different than vehicle control values.

Results of ANOVA for rimonabant, LY379268, MPEP, and MTEP are provided in the results section. One-way ANOVA results for the other compounds in this table are as follows:

Chlorpromazine: Reinstatement [F (1, 16) = 0.64, $p > 0.05$]; FR1 [F (1, 14) = 0.79, $p > 0.05$]

Chlordiazepoxide Reinstatement [F (1, 16) = 0.48, $p > 0.05$]; FR1 [F (1, 14) = 0.62, $p > 0.05$]

ABT431: Reinstatement [F (1, 14) = 0.37, $p > 0.05$].

d-Amphetamine: Reinstatement [F (1, 14) = 0.45, $p > 0.05$]; FR1 [F (1, 16) = 4.2, $p < 0.05$]

Naltrexone: Reinstatement [F (1, 14) = 0.29, $p > 0.05$]; FR1 [F (1, 14) = 0.41, $p > 0.05$].

Citalopram: Reinstatement [F (1, 16) = 0.61, $p > 0.05$].

FIGURE LEGENDS

Figure 1. Effect of various stimuli on the ability to reinstate responding after extinction of food maintained responding. Each bar represents the mean (\pm SEM) of 5-9 rats/group. * $p < 0.05$ compared to data of comparable group on the last day of extinction (control: C1-C6).

Figure 2. Acquisition, extinction, and reinstatement of food-maintained responding in three separate group of rats. Each point represents the mean (\pm SEM) of 5-9 rats/group. On day 8, responses produced in separate groups of rats either food alone (filled circle), food + food-associated stimuli (unfilled circle), or food-associated stimuli alone (triangle). # $p < 0.05$ compared to data of comparable group on the last day of response acquisition. * $p < 0.05$ compared to the last day of extinction.

Figure 3. Top Panel: Effect of MTEP (3.0 – 10.0 mg/kg) on the reinstatement of food-maintained responding (black bars) or on the maintenance of responding under the FR1 schedule (checkered bars). **Bottom Panel:** Effect of MPEP (3.0 – 10.0 mg/kg) on the reinstatement of food-maintained responding (black bars) or on the maintenance of responding under the FR1 schedule (checkered bars). All data are shown as mean (\pm SEM) of 5-9 rats/group. * $p < 0.05$ compared to vehicle with Dunnett's post-hoc.

Figure 4. Top Panel: Effect of LY379268 (1.0 – 5.6 mg/kg) on the reinstatement of food-maintained responding (black bars) or on the maintenance of responding under the FR1 schedule (checkered bars). **Bottom Panel:** Effect of rimonabant (10.0 mg/kg) on the reinstatement of food-maintained responding (black bars) or on the maintenance of responding under the FR1 schedule (checkered bars). All data are shown as mean (\pm SEM) of 5-9 rats/group. $p < 0.05$ ** $p \leq 0.01$ compared to vehicle with Dunnett's post-hoc.

Figure 1.

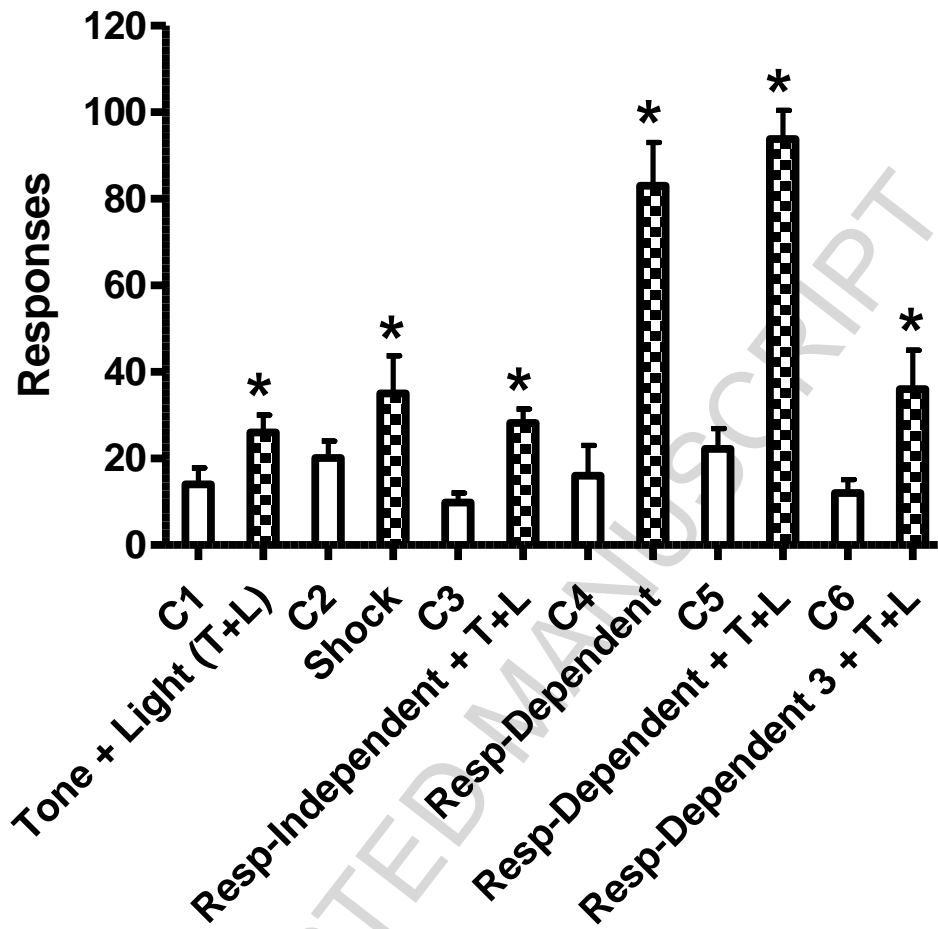


Figure 2.

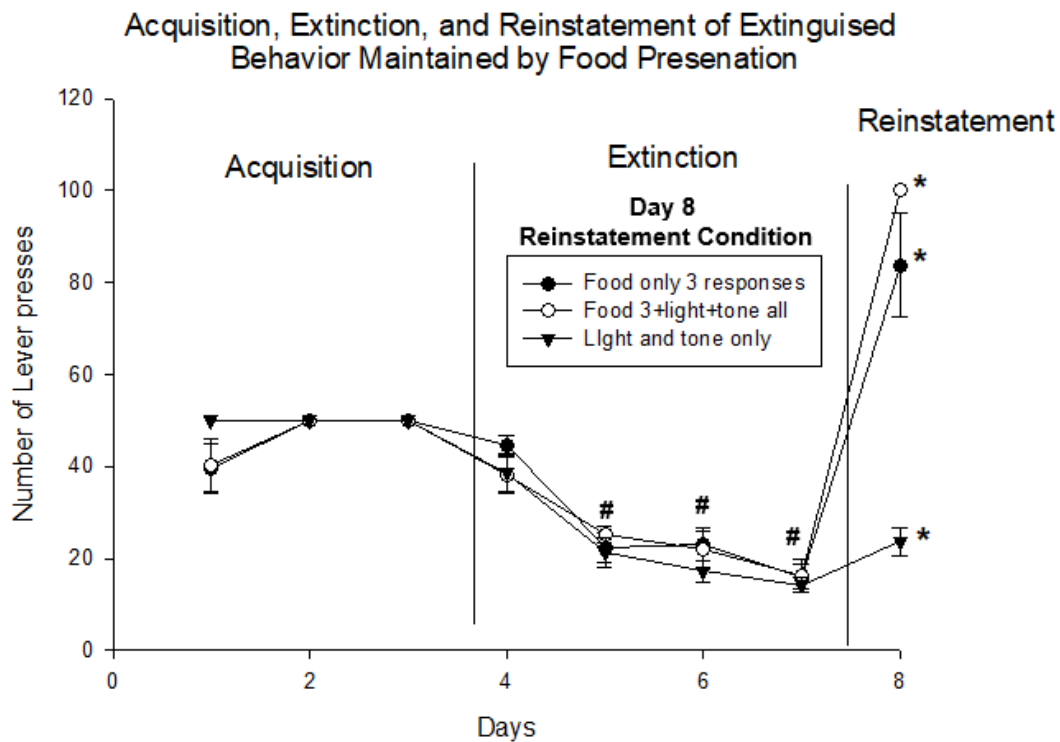


Figure 3.

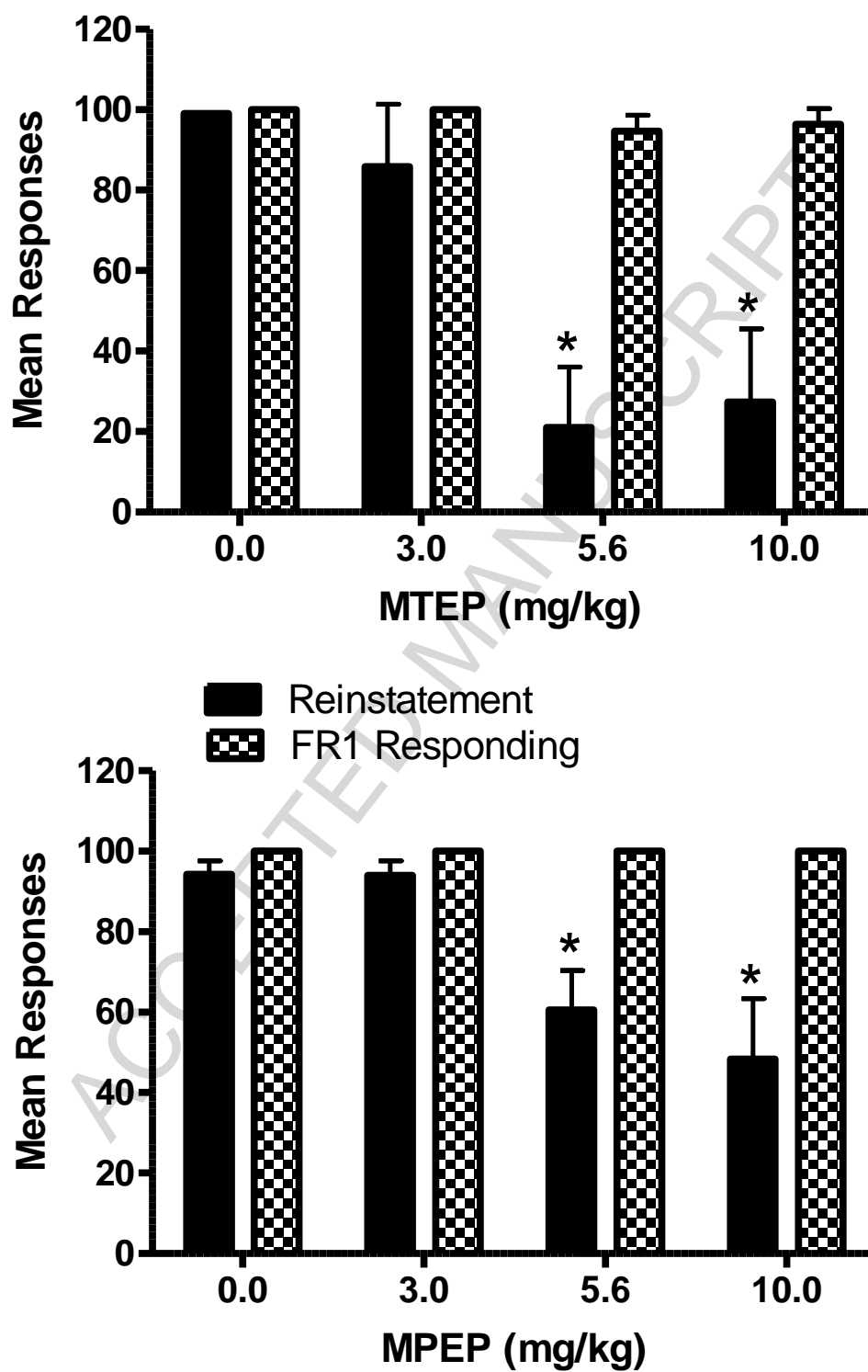
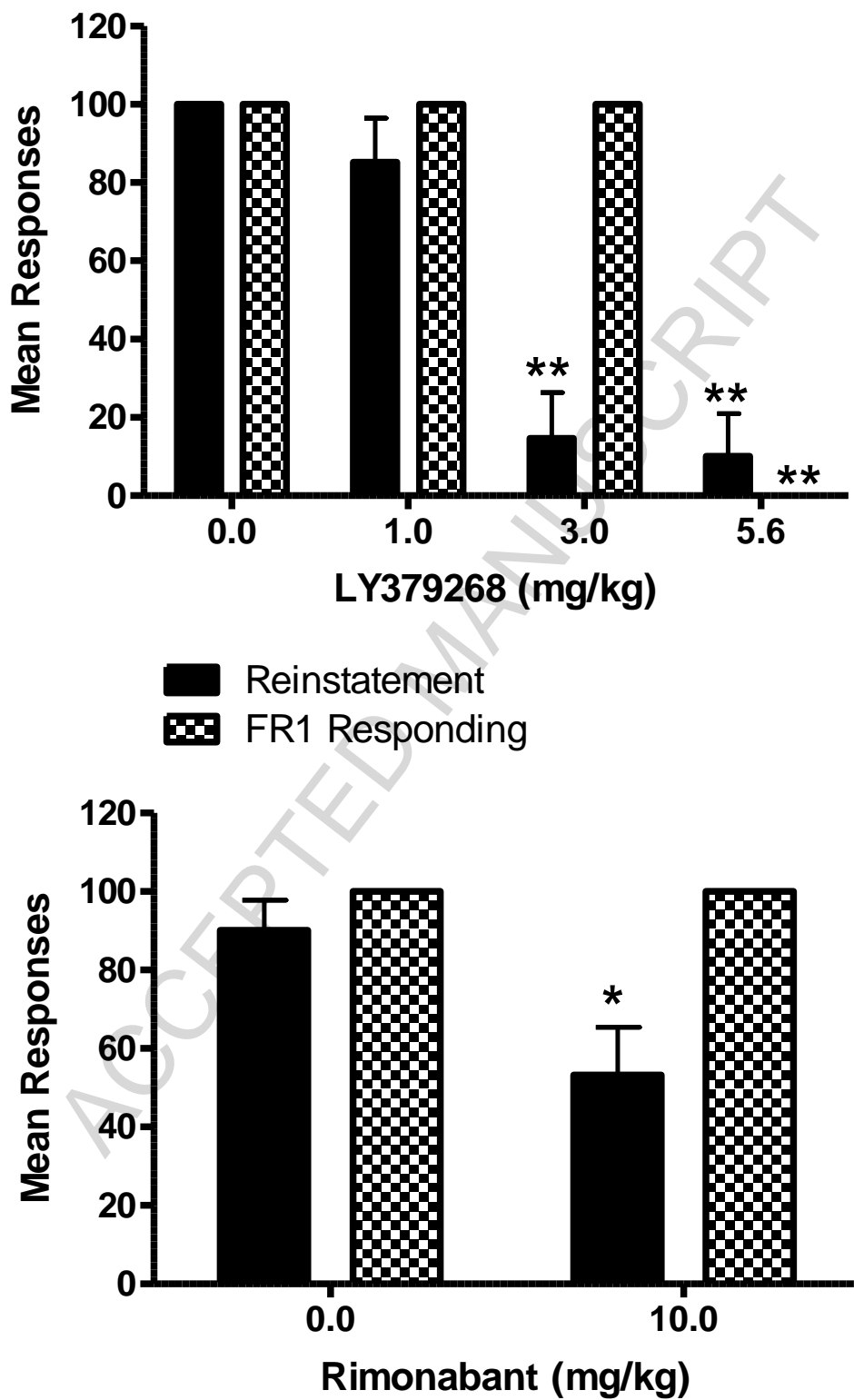


Figure 4



A medium throughput rodent model of relapse from addiction with behavioral and pharmacological specificity

Highlights

An 8-day rat model of reinstatement is presented using food reinforcement

Rimonabant, LY379268, MTEP, and MPEP suppressed reinstatement

Reinstatement was suppressed at doses without effect on ongoing behavior

Clozapine, *d*-amphetamine, chlordiazepoxide, ABT-431, naltrexone and citalopram were inactive

The model might be useful for screening compounds for addiction-associated relapse