Video Article

A Conflict Model of Reward-seeking Behavior in Male Rats

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Abstract

The present protocol describes a novel conflict task as a model of inhibitory control in rats. In this model, a natural rewarding stimulus (sexual stimulus) that represents a high-value reward, and the aversive stimuli (pins), are concurrently presented. The male rats have to climb or jump over the obstacle full of pins to approach and investigate the sexual partner. If the animal persists in their approaching behavior regardless of the aversive stimuli, it is considered as a maladaptive or risky reward-seeking behavior. The conflict task permits the evaluation of deficit in inhibitory control resulting from exposure to abused drug, such as morphine, or a stressful event.

The main advantage of this model is that it provides a simple and quick way to discover the deficit in inhibitory control after exposure to opiate drugs or other stressful events. In addition to opiates, this behavioral model would also be useful for quickly discovering the inhibitory control deficits induced by other addictive drugs. However, the limitation is that the male rats' performance may be subject to exercising effects with repeated testing under this conflict task. In the future, one can hope that the individuals with the compulsive phenotype of reward-seeking behavior after exposure to opiates will be identified based on modifying this conflict model.

Introduction

Drug addiction is a chronic brain disease which is characterized by impulsive and compulsive drug seeking and taking[1]. These key features of addiction have both been hypothesized to result from the impaired ability of inhibitory control[2,3], i.e., failing in inhibiting the immediate pursuit of rewarding stimuli and thus developing maladaptive patterns of behavior[4].

The go/no-go task and stop-signal task are the prototypical tasks used to measure the ability of response inhibition[2,5]. These two experimental paradigms assess one's ability to suppress actions that are inappropriate, by contrasting infrequent inhibitory responses against an implicit go baseline[6,7]. The response inhibition displayed in these tasks has been shown to be impaired in cocaine users[8,9], opiate addicts[10], and nicotine users[11]. Another two tasks—reversal learning and multiple choice serial reaction time tasks—also provide measurements of response inhibition/inhibitory control[12,13]. However, most of these paradigms performed in rodents not only require long-term training so that subjects can distinguish the response requirements represented by different signals, but also the individual differences in learning speed and learning effects may interfere with the results of subsequent inhibitory test[11].

In this paper, we present a novel conflict task which can be used to measure the impairment of inhibitory control after exposure to addictive drugs. In this task, a natural rewarding stimulus (sexual stimulus) that represents a high-value reward[14], and the aversive stimuli (pins) that male rats have to conquer, are concurrently presented. The male rats have to climb or jump over the obstacle full of pins to approach and investigate the sexual partner. If the animal persists in its approaching behavior regardless of the aversive stimuli, it is considered as a maladaptive or risky reward-seeking behavior. One of the rationales for establishing this task is that it is conceptually simple and does not place heavy demands on executive processes as other tasks do. Compared to other tasks which measure response inhibition, this conflict task is based on natural behavior, and rats with normal sexual function and sexual experience can be tested directly without a learning process. Another rationale is that a conflict presented in this task between approaching the reward and avoiding the aversive stimuli (or the risk of being pricked) may have a better validity, as it mimics what occurs in addicts who often place themselves in the similar conflict but persistently pursue drug reward regardless of the risk of negative consequences in real life[15].

Therefore, application of this conflict model is a quick and sensitive way to discover the deficit in inhibitory control after exposure to addictive drugs, or other factors that may influence ability of inhibitory control, such as stress. It also provides a novel behavioral strategy for investigation of neural mechanisms underlying deficits in inhibitory control. Furthermore, alternative modifications can be added onto this task. For example, altering the cost/benefit ratio by replacing the sexual stimulus with the social stimulus can reveal more behavioral significances.

Protocol

This study is approved by the International Review Board (IRB) of the Institute of Psychology, Chinese Academy of Sciences, and all experiments are conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

1. Materials and setup for the conflict model

1. House four rats per cage (50 cm long x 22.5 cm wide x 30 cm high) in colony rooms with controlled temperature (22–25 °C) and a reversed 12 h/12 h light/dark cycle (lights on at 21:00) for at least 10 days.
   NOTE: Male and female Sprague-Dawley rats weighing 330–400 and 230–250 g at the beginning of the experiments, respectively were used.
2. Perform all the tests under dim lighting during the dark phase.
3. Use the opaque cartons (37 cm long x 26 cm wide x 18 cm high) with pine wood shaving bedding to transport rats from home cages to testing rooms.
4. Handle rats 3 min each per day for 5 days prior to beginning the experiments.
5. Prepare 120 mL of morphine for the whole experiment. Dissolve morphine hydrochloride in 0.9% NaCl (physiological saline) at a final concentration of 20 mg/mL. Store at room temperature (RT).
6. Prepare 10 mL of the pentobarbital sodium solution for the surgery. Dissolve pentobarbital sodium in 0.9% NaCl at a concentration of 1 g/mL. Store at 4 °C.
7. Prepare 10 mL of estradiol benzoate (EB) and 10 mL of progesterone for artificial induction of estrus. Dissolve estradiol benzoate and progesterone in sesame oil at a concentration of 0.125 mg/mL and 5 mg/mL, respectively.
   NOTE: Incubate the oil suspension of EB or progesterone in a water bath (55–60 °C) for at least 1 h and then thoroughly shake it. Ensure that EB or progesterone is dissolved completely. Store at RT.
8. For the risky reward-seeking behavior testing, use the open-field reward-proximity chambers made of black acrylic glass. At one end of the open-field arena (85 cm long x 35 cm wide x 50 cm high) mount a wire-screen stimulus cage (15 cm long x 25 cm wide x 25 cm high). Install the board with pins on the floor about 20 cm in front of the stimulus-cage.
   NOTE: Pins are fixed on the 34.5 cm long x 13 cm wide boards (**Figure 1**). The boards with three types of pins are used in turn during testing (**Table 2**).

2. Estrus induction in females and mating screening in males

1. **Bilateral ovariectomy in female rats**
   1. Prepare surgical instruments and materials such as scalpels, surgical blades, hemostatic forceps, tweezers, ophthalmic scissors, gauze, cotton swabs, suture needles and silk sutures in advance, as well as 75% alcohol, iodine, sodium pentobarbital, 0.9% NaCl, penicillin sodium.
   2. Intraperitoneally inject pentobarbital sodium (55 mg/kg), waiting for complete anesthesia of female rats. Rats respire smoothly and do not react to tail pinch.
      NOTE: The ovariectomy is performed only when the weight of female rat reaches at least 240 g.
   3. Place the female rat in prone position, shave off the fur on the back, and disinfect the exposed skin with iodophor. Then use a scalpel to make a middle vertical incision (about 2 cm long) on the back (1 cm below the edge of the ribcage).
   4. Pull the skin to the left side, and blunt dissect subcutaneous tissue with hemostatic forceps to expose the lumbar muscles. Cut the muscle layer (incision of 1 cm) into the abdominal cavity, till the adipose tissue is reached.
   5. Pull out the adipose tissue with bent tweezers, and locate the ovary which is a flesh-pink tissue (about 0.5 cm x 0.4 cm x 0.3 cm) surrounded by adipose tissue with the twining fallopian tube on the surface.
   6. Clamp the fallopian tube with forceps and ligate it, then cut off the ovary together with the surrounding adipose tissue. Disinfect the residual fallopian tube with iodophor.
   7. Put the residual fallopian tube and adipose tissue back into the abdominal cavity after ensuring no bleeding, and suture the muscle layer.
   8. Remove the ovary on the other side by the same procedure and then suture the skin incision. Disinfect the incision again with iodophor. Inject 200,000 U of penicillin sodium into the hind leg muscle.
   9. Place the rat on a heated blanket till awakening and then put it back into the home cage.
   10. After at least two weeks of recovery, use the female rats as a tool for mating screening and behavioral testing.
       NOTE: Inject 200,000 U of penicillin sodium for three consecutive days after surgery, and supply adequate water and food. Keep the ovariectomized rats single housed for one week and then house four per cage.

2. **Induction of estrus in ovariectomized female rats**
   1. Handle all ovariectomized female rats three times (3 min/day) before using them for mating screening.
      1. Gently pick up a rat from the transport box with the left hand and hold it in the arms for a few seconds. Then put the rat back into the box and repeat these operations for 3 min.
   2. Subcutaneously inject estradiol benzoate (25 μg/rat) about 48–52 h before mating screening or the conflict test.
   3. Subcutaneously inject progesterone (1 mg/rat) about 4–6 h before mating screening or the conflict test.
      NOTE: The estrogenic hormones are injected subcutaneously on the back of the neck. Since an estrus cycle lasts for ~4–5 days, the female rats are used once a week.

3. **Screening male rats for mating performance**
   NOTE: Screening is conducted under dim light during the dark phase of the light/dark cycle in the housing room.

1. Place a male rat individually into the carton (60 cm long x 50 cm wide x 40 cm high) with pine wood shaving bedding and leave it to habituate for 5 min.
2. Introduce an estrous female rat into the carton and monitor male copulatory behaviors (by experienced observers).
3. Put the rats back to home cages after male rats complete their first ejaculation within 30 min or do not display intromission within 15 min or ejaculation within 30 min.
4. Assign the male rats that pass the screening (successful ejaculation for three consecutive days) randomly into different groups (such as the saline- and morphine-treated groups).

3. Pretreatment in male rats prior to the conflict test

1. **Binge-like morphine treatment**
   NOTE: Male rats are intraperitoneally injected with saline or morphine delivered in a binge-like regimen[14] (**Table 1**).
   1. Weigh the male rats and calculate the injection volume for each rat based on the body weight (see **Table 1**).
   2. Prepare syringes with morphine or saline solutions.
   3. Inject one rat at a time intraperitoneally and immediately place it gently into the home cage (4 rats/cage).
   4. After at least 6 h, give the male rats the second injection in the same way.

2. **Acute stress**
   NOTE: Foot shocks are delivered prior to each conflict test in four identical chambers assembled with four shock generators and controlled by professional software installed on a computer.
   1. On the day of the conflict test, take the male rats to another room different from the conflict testing room.
   2. Put the male rats into the chambers (30.5 cm long x 25.4 cm wide x 30.5 cm high) to habituate for 1 min.
   3. Set up the software program in advance. The program includes the intermittent foot shocks delivered within 10 min (0.5 mA x 0.5 s x 10 min; mean inter-shock interval 40 s, range 10–70 s).
   4. Enter animal IDs and choose whether to turn on the shock generators according to the grouping (the shock group and the control group). Then press the **start** button.
   5. When the stress procedure is finished, bring the rats immediately to the conflict test room in transport boxes; one rat per box.

4. The conflict test

NOTE: The test is conducted under dim light during the dark phase of the light/dark cycle in the conflict test room.

1. On the day before testing, bring all rats to the conflict test room and allow them to habituate to the open-field reward-proximity chamber (without any obstacle, **Figure 1**) for 15 min.
2. On the testing day, place the male rat in the chamber allowing free exploration for 10 min (under the same condition as the day before testing).
3. Place an estrous female rat in the stimulus-cage as an incentive and allow the male subject to freely approach and investigate the incentive rat for 5 min.
4. After 5 min-free approach, move the male subject from the stimulus-cage to the other end of the arena, place an obstacle (a 14 cm-wide board thick with pins), and then start the first trial of the test.
   NOTE: The difficulty level of obstacles is varied among trials based on the types of pins and the height of the board. The grading system is shown in **Table 2**.
5. Move the male rat away from the stimulus-cage about 15-20 s after each time it surmounts the obstacle.
6. End a trial if the male subject climbs or jumps over the obstacle 3 times within 4 min and immediately start the next trial with increasing difficulty of the obstacle.
7. If a male subject surmounts the obstacle less than three times within 4 min, end the test and record the times it surmounts the obstacle.
8. Bring the male rat back to the home cage and scrub the open-field chamber with 0.05% glacial acetic acid.
9. Score each surmounting (or approaching) as per **Table 2**. Use the sum of the scores for all of the surmountings as a total score for a male subject in this conflict test.

5. Statistical analysis

1. Present the data as mean ± SEM or single data points. In case that homogeneity of variance or normal distribution of the datasets is challenged, log-transform the data sets.
2. Analyze the effects of morphine pretreatment on reward-seeking behavior displayed on day 7 and day 17 of withdrawal (Wd7 and Wd17) using t-tests with “pretreatment” as a between-subjects factor (morphine versus saline, **Figure 2**).
3. Analyze the effects of morphine pretreatment on reward-seeking behavior after repeated testing using repeated-measure’s analysis of variance (ANOVA) with “withdrawal time” (Wd7 versus Wd14) as the within-subjects factor and “pretreatment” (morphine versus saline) as a between-subjects factor.
4. In addition, use Pearson’s correlation to analyze the correlation between the scores that male subjects acquired on days 7 and 14 of abstinence (Wd7 and Wd14, **Figure 3**).
   NOTE: After the logarithmic conversion of the original data, statistical analyses are performed.
5. Analyze the effect of acute stress on reward-seeking behavior in drug-naïve rats using t-test with stress as a between-subjects factor (shock versus control, **Figure 4**)

Representative Results

To explore whether this conflict model can reveal maladaptive/risky reward-seeking behavior induced by opiates, the reward-seeking behaviors displayed by the saline- and morphine-pretreated groups were compared by t-tests after short-term (Wd7) and long-term (Wd17) withdrawal from morphine respectively (**Figure 2**). The results show that on both day 7 and day 17 of withdrawal, the morphine-pretreated rats showed significantly more approaching behaviors than the saline-pretreated rats (**Figure 2a**: t = -3.958; d.f. = 24; $p < 0.01$. **Figure 2b**: t = -2.350; d.f. = 17; $p < 0.05$), suggesting that the morphine-withdrawn rats displayed more perseverative behaviors in the face of the aversive obstacle and this maladaptive behavior persists for a long time after withdrawal.

When the rats were repeatedly tested under the conflict task (**Figure 3**), the repeated-measure ANOVA showed a significant main effect of Pretreatment (F (1,24) = 12.910; $p < 0.01$). Neither significant effect of Withdrawal time (comparing Wd7 and Wd14, [F (1,24) = 0.807; $p > 0.05$]) nor significant interaction (Withdrawal x Pretreatment interaction: F (1,24) = 1.093, $p > 0.05$) was found (see **Figure 3a**). To further investigate the stability of the risky reward-seeking behavior, the correlation of the scores between different withdrawal periods (between Wd7 and Wd14) was analyzed. The results showed that the approaching behaviors during short- and long-term withdrawal periods were significantly correlated (Pearson correlation: r = 0.445; $p < 0.05$, see **Figure 3b**), indicating that the rats' risky reward-seeking behaviors were roughly stable over repeated tests.

To investigate the influence of stressful events on the ability of inhibitory control in animals, the drug-naïve rats were exposed to an intermittent foot-shock stress before the conflict test. Although there was no significant difference between the stress and control groups (**Figure 4a**: t = -1.207; d.f. = 17; $p > 0.05$), the reward-seeking behaviors displayed a bi-modal distribution within the stress group, suggesting the markedly differential effects of acute stress on risky reward-seeking behaviors among individuals (see **Figure 4b**).

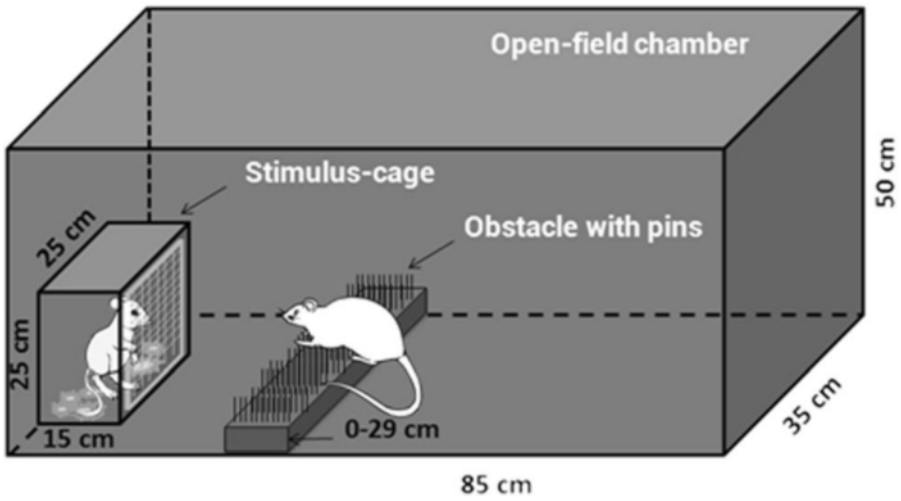


Figure 1: The apparatus for the conflict test. The open-field chamber (85 cm long x 35 cm wide x 50 cm high) with a stimulus cage holding an estrous female rat was used for testing the reward-seeking behaviors under conflict. The male subjects had to surmount a dangerous obstacle, i.e., climbing over a continuously heightened board (34.5 cm long x 13 cm wide) thick with pins, to approach the stimulus cage. Please click here to view a larger version of this figure.

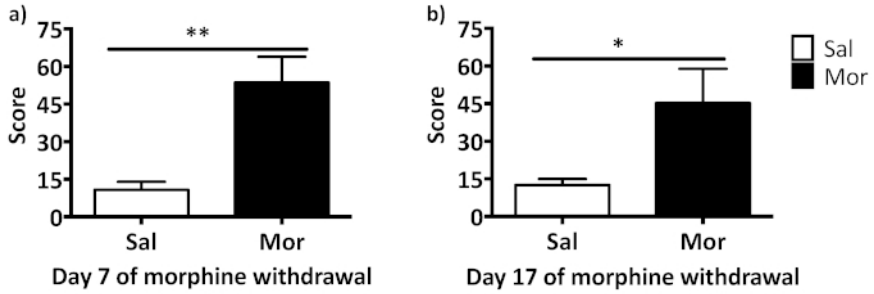
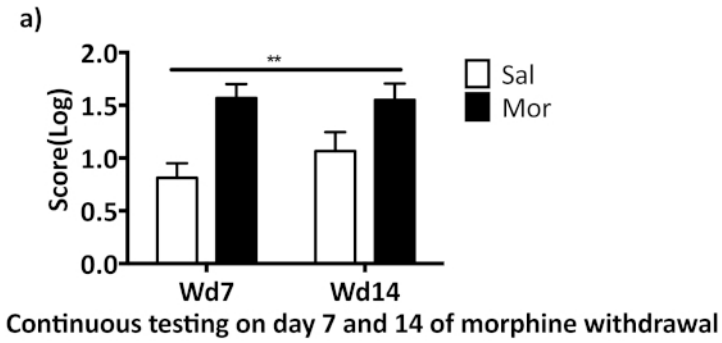


Figure 2: The risky reward-seeking behaviors in the conflict tests displayed by male rats. (**a**) The scores for approaching behaviors displayed by the morphine- (black) and saline- (white) treated groups on day 7 of withdrawal. (**b**) The scores for approaching behaviors displayed by the morphine- (black) and saline- (white) treated groups on day 17 of withdrawal. The bars represent mean ± SEM. * indicates $p < 0.05$; ** indicates $p < 0.01$; Sal = Saline, Mor = Morphine.This figure has been modified from[17] with permission. Please click here to view a larger version of this figure.



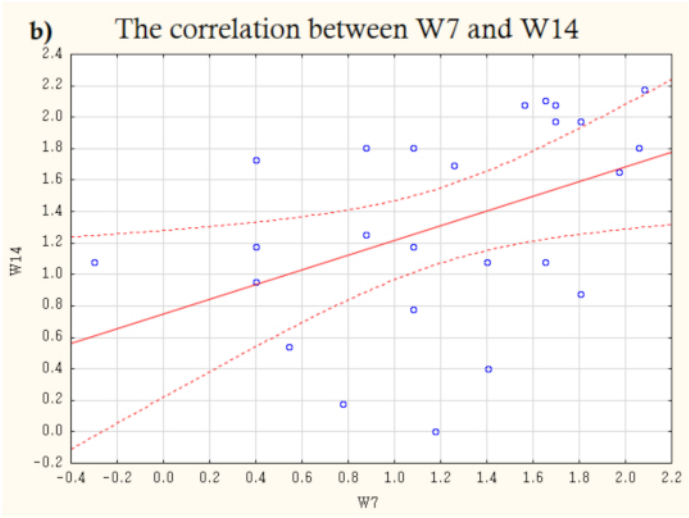


Figure 3: The risky reward-seeking behaviors consecutively tested on day 7 and 14 of withdrawal from morphine. (**a**) The scores for approaching behaviors displayed by the morphine- (black) and saline- (white) treated groups on day 7 and 14 of withdrawal (Wd7 and Wd14). The bars represent mean ± SEM. (**b**) The correlation of the scores for risky reward-seeking behaviors between two tests on day 7 and 14 of withdrawal. * indicates $p < 0.05$; ** indicates $p < 0.01$. Sal = Saline, Mor = Morphine. Please click here to view a larger version of this figure.

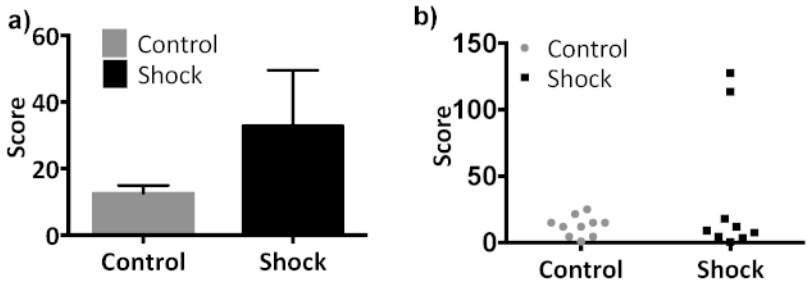


Figure 4: Effect of the foot-shock stress on reward-seeking behaviors in the conflict test. (**a**) The scores for approaching behaviors displayed by the control (gray) and shock (black) groups subjected to saline pretreatment. The bars represent mean ± SEM. * indicates $p < 0.05$; ** indicates $p < 0.01$. (**b**) The scores for approaching behaviors displayed by the control and shock groups are shown in single points. These figures have been modified from[17] with permission. Please click here to view a larger version of this figure.

	Day 1	Day 2	Day 3	Day 4	Day 5
1st injection	10 mg/kg	20 mg/kg	40 mg/kg	40 mg/kg	40 mg/kg
2nd injection (6h later)	20 mg/kg	40 mg/kg	40 mg/kg	40 mg/kg	40 mg/kg

Table 1: Binge-like morphine treatment regimen. Male rats are pretreated twice daily for 5 days with intraperitoneal injections of either saline or morphine delivered in a binge-like regimen[17]: 10, 20, 20, 40, 40, 40, 40, 40, 40, and 40 mg/kg. The two doses of morphine administered on each day are at least 6 h apart.

	Day 1	Day 2	Day 3	Day 4	Day 5
1st injection	10 mg/kg	20 mg/kg	40 mg/kg	40 mg/kg	40 mg/kg
2nd injection (6h later)	20 mg/kg	40 mg/kg	40 mg/kg	40 mg/kg	40 mg/kg

Table 2: The grading for the amount of difficulty the subject conquered per approach. According to the length of pins and average distance between pins, three types of board thick with pins were used: *a* with pin-length of 0.5 cm, average distance of 1 cm; *b* with pin-length of 0.8 cm, average distance of 0.5 cm; and *c* with pin-length of 2 cm, average distance of 1 cm. The board was repeatedly heightened as follows: 0, 2, 4, 7, 10, 13, 17, 21, 25, and 29 cm. Thus, there are 12 levels of difficulty of surmounting the obstacle, i.e., 12 trials, during the test. The amount of difficulty the subject conquered each time to approach the stimulus cage is scored and summed up to the total score for each subject[17].

Discussion

The inhibitory control deficits caused by drug abuse[18] play a key role in promoting compulsive drug seeking/taking behaviors and relapse[19,20]. The conflict model presented here provides a new approach to explore the changes in inhibitory control of the individuals exposed to addictive drugs.

There are several critical steps in the protocol. First of all, the subjects (male rats) must acquire sexual experience before entering the follow-up conflict task. For example, the male rats need to pass the screening for copulation (copulating at least three times) before drug treatment, and are assigned into different groups randomly. In order to carry out the copulation screening smoothly, two points should be paid attention to. One is the body weight of female rats upon ovariectomy. The ovariectomy is performed only when the weight of female rats reaches at least 240 g. Since the body maturation of female rats occurs later than gonadal maturation[21], early ovariectomy could hinder male rats from successfully copulating with female rats even though the females were administered estrogenic hormones. The other point is that male rats should habituate to the screening boxes before copulation with female rats, in order to avoid the influence of neophobia to a novel context on following copulation.

Stress factors should be tightly controlled throughout the experiment. Both male and female rats are handled and habituated to transportation before the conflict task starts. Moreover, during the habituation to the reward-proximity chamber before the testing day, male rats are repeatedly grasped and moved from the front of the stimulus cage to the other end of the chamber several times.

As the conflict task continues, the obstacle (the board with pins) is repeatedly heightened, thus male rats behind the obstacle cannot see or directly feel the female rat in the stimulus cage any more. In order to keep the male rats attracted continuously by the sexual partner, some bedding material soiled by estrous female rats are placed in the stimulus cage, allowing the female odor to diffuse in the air. The beddings are collected ahead of the conflict task and stored at -20 °C until use.

In this conflict task, one can also investigate the impact of reward value on reward-seeking behaviors, for example, changing the reward value by replacing the female rat (sexual reward) with a male rat (social reward)[14,16]. If both sexual and social rewards are used in one experiment, it would be best to run the experiment in two reward-proximity chambers, one for sexual reward and the other one for social reward. If the experiment has to be carried out in one chamber, both the chamber and the stimulus cage must be carefully cleaned with 0.1% glacial acetic acid between subjects as well as tests, in order to avoid the olfactory cross-contamination between male and female stimuli. The pins on board should be renewed regularly to ensure their constant threat to male rats.

The male rats' performance may be subject to exercising effect with repeated testing under this conflict task. We noticed that some animals (especially the saline-pretreated rats) could avoid the pins better through exercising on jumping over the obstacle during the second test (**Figure 3a**). Although there is a significant correlation between the risky reward-seeking behaviors during the two repeated tests, it has not been verified if this task can be used for identifying the high- and low-risk phenotypes of reward-seeking behavior. Hence, so far this conflict task is suited to quickly probe any changes in inhibitory control after pharmacological or behavioral treatments.

Compared with go/no-go task, stop signal task, multiple choices serial reaction time task and reversal learning, which reflect the subject's ability of inhibition on the inappropriate responses after learning the rules of tasks[2,5], in this conflict task, the subjects do not need to inhibit any learned responses but inhibit their spontaneous craving/motivation due to the possible negative consequence. Hence this conflict task better mimics the conflict situation which addicts often face and helps to examine the ability of inhibitory control during the psychological process of weighing up the cost and benefit. Moreover, in contrast with other decision-making tasks, such as risky decision making[22,23] or gambling task[24,25], this conflict task is simpler and easier to perform, since it only consists of single reward and single risk.

The conflict model we established can be used to reveal deficits in inhibitory control after exposure to the opiate drug—morphine. We believe that this behavioral model would also be useful for quickly discovering the impairment of inhibitory control induced by other addictive drugs. Furthermore, the reliable opiate-induced compulsive reward-seeking behavioral phenotype will be identified based on this conflict model. At present, we are working to replace the obstacle (the board full of pins) with an electric grid so that foot shocks can be delivered to male rats, and are also modifying the behavioral procedure for repeatedly measuring animal's reward-seeking behaviors in the face of the negative consequence (foot shocks). As is known, there is no available behavioral model of opiate-induced compulsive reward-seeking behavior so far, which is probably because of the analgesic effect of opiates[26,27].

Clinically, stress is one of the important factors leading to relapse after drug withdrawal[28]. In addition, stress also is an important influencing factor for behavioral inhibition/impulsivity/compulsivity[29]. The introduction of this conflict model also allows us to observe the influences of various stressful events on the ability of inhibitory control in subjects quickly following exposure to abused drugs.

Disclosures

The authors have nothing to disclose.

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