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# The expression of morphine-induced behavioral sensitization depends more on treatment regimen and environmental novelty than on conditioned drug effects

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Both conditioned responses (CRs) and sensitized behaviors induced by addictive drugs are considered to reflect drug-seeking motivation. Based on an excitatory conditioning model of behavioral sensitization, this work hypothesizes that conditioned locomotor activity and locomotor sensitization concomitantly occur using different drug treatment regimens. In the present study, conditioned locomotor activity and sensitized locomotion and stereotypy are assessed with pretreatment of two doses of morphine in a familiar or novel environment. When rats are trained with morphine (3 or 5 mg/kg) in an environment to which the animals are habituated, a CR but not contextual sensitization is induced when tested after 1 week of abstinence. When rats receive the 5 mg/kg dose of morphine immediately after placement into a novel environment, the same results are obtained, but when the drug dose is decreased to 3 mg/kg, both the CR and contextual sensitization are observed. Therefore, the sensitized behaviors, rather than the CR produced by morphine pretreatment, appear to be dependent on the drug treatment regimen and environmental novelty, suggesting that different mechanisms may be involved in the expression of the CR and contextual sensitization.

**morphine, conditioning, sensitization, locomotion, stereotypy**

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Repeated intermittent exposure to potentially addictive drugs leads to pathological levels of incentive salience attributed to drugs and drug-associated cues [1,2]. According to the excitatory conditioning model, sensitization is context-specific because it occurs in response to Pavlovian conditioning [3,4]. However, dissociation between the conditioned response (CR) and behavioral sensitization has been found in many studies, e.g. amphetamine and methylenedioxymethamphetamine (MDMA) induced conditioned hyperlocomotion in the absence of prior sensitization [5,6]. By contrast, extinction of the post-sensitization CR did not impede the subsequent expression of contextual sensitization [7,8]. The behavioral effects induced by frequently abused drugs are significantly impacted by the drug dose and the experimental drug administration design [9–12]. The present study sought to verify the relationship between CR and drug-induced sensitized behaviors using different morphine-treatment regimens by altering the drug doses and the environment novelty.

In the present study, when rats were trained with morphine (5 mg/kg) in an environment to which the animals were habituated, a CR occurred, but not contextually sensitized behaviors, when tested after a 1-week abstinence period. The results were similar when the dose of morphine was reduced to a 3 mg/kg pretreatment. This diminished sensitization of locomotor activity did not derive from any "masking effect" of the emergence of stereotyped behavior. Despite the facilitating effect of a novel environment on

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behavioral sensitization [9], we were unable to observe sensitization of locomotor activity and stereotypy, with the exception of conditioned locomotion, when training with the 5 mg/kg of morphine was initiated in a novel environment. However, when a lower dose of morphine (3 mg/kg) was used during training, both the CR and behavioral sensitization were elicited.

## 1 Materials and methods

### 1.1 Subjects

Sixty-four male Sprague-Dawley rats (Vital River Animal Center, Beijing, China), weighing 230–250 g upon arrival, were used in this study. Rats were housed in groups of 4 in cages consisting of stainless steel sliding drawers (50 cm × 32 cm × 22 cm height) with a wire mesh front and floor and pine wood-shavings below the cages. Animals were maintained on a 12 h/12 h light/dark cycle (lights on at 07:00) with food and water available *ad libitum*. All rats were gently handled daily for 1 week to habituate them to handling before any experimental treatment. The experimental procedures were approved by the International Review Board of the Institute of Psychology, Chinese Academy of Sciences, and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

### 1.2 Behavioral measurements

Eight identical plastic, black rectangular activity chambers (40 cm × 40 cm × 50 cm height) were used for the behavioral tests. The chambers were placed in a dimly lit room illuminated by four overhead 15 V projection lamps mounted 200 cm above the chambers. A video camera was suspended from the ceiling to record the horizontal movements of the animals using a video tracking system. Movements were analyzed by a computerized system. Stereotyped behaviors were later scored by an observer who was blind to the treatment conditions, using a rating scale (Table 1) similar to that described by Miller et al. [13] and Forster et al. [14]. All ratings were performed by the same observer. Stereotypy was rated over 2 min periods every 15 min following morphine or saline injection. A total of 16 scores over 240 min was quantified as the final score of stereotyped behaviors in each training session.

### 1.3 Procedure

In Experiment 1, behavioral sensitization of rats was assayed with pre-exposure to the test environment. A protocol was designed to produce habituation to the test environment using 2 pre-exposure sessions before the drug training session began (Figure 1(a)). The treatment schedule was separated into 3 phases. (1) Acclimation: all animals received two 3 h acclimation sessions separated by 48 h before morphine or saline training. (2) Training: 48 h after termination of the pre-exposure session, 2 groups of animals respectively received morphine (5 mg/kg, i.p.) or saline injections. Five training sessions occurred over 10 d with one session every other day. During each training session, the animals were transported from their home cage to the test chambers and allowed to further habituate to the chambers for 1 h. Animals were videotaped for 120 min following morphine/saline injection. (3) Test: after a 1-week withdrawal period, animals in each group were placed in the test chamber for a 10 min conditioning test. The conditioning test was followed by a challenge injection protocol using 5 mg/kg morphine to analyze the expression of context-specific sensitization for 4 h. To verify the effects of different doses of morphine on the relationship between conditioning effects and sensitized behaviors, we used a lower morphine dose (3 mg/kg) for each morphine training session [15]. During the challenge test, rats received 3 mg/kg morphine (i.p.).

Experiment 2 probed the behavioral sensitization of rats with no exposure to the test environment. Rats received the morphine treatment in a novel environment without any pre-exposure or habituation to the test chambers (Figure 2(a)). Two groups of animals received either saline or morphine (3 or 5 mg/kg, i.p.) injections immediately prior to placement in the test chamber. Each group received five 2-h

**Table 1** Stereotypy rating scale

| Score | Behavior | Description |
|---|---|---|
| 0 | Inactivity | Lying down with eyes open or closed |
| 1 | Little activity | Locomotion for less than 1 min, with sporadic grooming or rearing |
| 2 | Normal activity | Locomotion for more than 1 min, with sporadic grooming or rearing |
| 3 | Intermittent non-oral-based stereotypy | Repetitive head-bobbing, grooming with limbs, and rearing for less than 1 min |
| 4 | Widely continuous non-oral-based stereotypy | Repetitive head-bobbing, grooming with limbs, and rearing for more than 1 min over a wide area |
| 5 | Restricted continuous non-oral-based stereotypy | Repetitive head-bobbing, grooming with limbs, and rearing for less than 1 min in restricted area |
| 6 | Oral-based stereotypy directed at self | Repetitive licking, biting, and grooming with mouth in a restricted area |
| 7 | Intermittent oral-based stereotypy directed externally | Repetitive licking or biting of walls and floor for less than 1 min |
| 8 | Continuous oral-based stereotypy directed externally | Repetitive licking or biting of walls and floor directed externally for more than 1 min |
| 9 | Dyskinesia | Abnormally maintained postures or dyskinetic movements |

training sessions with one training session every other day. After a 1-week withdrawal period, animals were tested to evaluate conditioning and sensitization effects induced by repeated morphine-environment pairings. The test procedure was the same as that of Experiment 1.

To minimize the association between morphine conditioning and the injection schedule, all rats in Experiments 1 and 2 received a saline injection in their home cage 2 h after each training session. Although stereotypy is typically elicited by high doses of morphine in rats [16,17], some investigators suggest that a low dose of morphine (2 and 4 mg/kg) also generates marked stereotyped behaviors [14,18]. Therefore, in addition to quantifying locomotor activity, the stereotyped behaviors of the animals were also rated.

### 1.4 Drugs

Morphine hydrochloride (Qinghai Pharmaceutical Co. Ltd, Qinghai, China) was dissolved in 0.9% saline to a final concentration of 2 mg/mL. Both morphine and saline were intraperitoneally administered (i.p.).

### 1.5 Data analysis

Data were processed using Graph Pad Prism 4.0 software (San Diego, CA, USA). Results were analyzed using Student's *t*-test or one-way analysis of variance (ANOVA) followed by Newman-Keuls *post hoc* test. In all tests, the criterion for significance was $P<0.05$.

## 2 Results

Figure 1 presents the schedule and the results of Experiment 1. During morphine/saline training, rats underwent complete inter- and intra-session habituation to the test environment. After a 1-week withdrawal period, conditioning effects and morphine-induced contextual sensitization were evaluated. One-way ANOVA revealed that rats subjected to morphine-environment pairings exhibited conditioned hyperlocomotor activity as contrasted with rats that received saline-environment pairings ($F_{2,29} = 7.39$, $P = 0.0039$). However, in the subsequent challenge test, we did not find any behavioral sensitization with 5 mg/kg morphine pre-treatment (Sal-Mor group *vs.* Mor-Mor group; locomotion: $t_{14} = 0.19$, $P = 0.8557$; stereotypy: $t_{14} = 0.66$, $P = 0.5179$) or 3 mg/kg morphine pretreatment (Sal-Mor group *vs.* Mor-Mor group; locomotion: $t_{14} = 0.90$, $P = 0.3791$; stereotypy: $t_{14} = 0.84$, $P = 0.4165$).

Figure 2 shows the schedule and results of Experiment 2. Compared with Experiment 1, a procedural distinction in Experiment 2 was that morphine or saline training was initiated in a novel environment. One-way ANOVA revealed that after a 1-week withdrawal period, significant conditioning effects were observed in the 2 morphine training groups ($F_{2,29} = 10.74$, $P = 0.0001$). Rats that underwent morphine (3 mg/kg)-environment pairings exhibited higher locomotor activity (Student's *t*-test, $t_{14} = 2.35$, $P = 0.0369$) and stereotypy ($t_{14} = 4.95$, $P = 0.0003$) scores compared with saline-environment pairings when the animals received an acute 3 mg/kg morphine injection. By contrast, with the

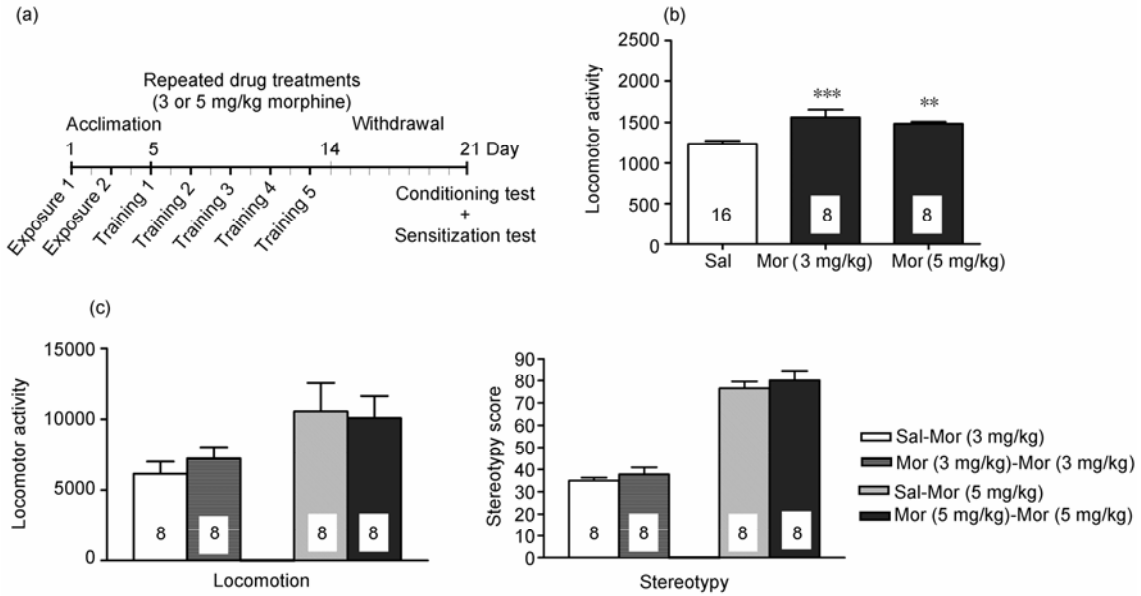


**Figure 1** Conditioned response and expression of sensitized behaviors in rats with previous exposure to the test environment during training. (a) Experimental procedure. (b) Conditioned locomotion. $^{**}P<0.01$, $^{***}P<0.001$, compared with saline training group (one-way ANOVA followed by Newman-Keuls *post hoc* test). (c) Contextual sensitized behaviors challenged by morphine, including locomotor activity and stereotypy.

5 mg/kg morphine training using the same procedure, we did not observe greater behavioral sensitization than the marked acute effects induced by 5 mg/kg morphine (Sal-Mor group *vs.* Mor-Mor group; locomotion: $t_{14} = 0.19$, $P = 0.8557$; stereotypy: $t_{14} = 1.07$, $P = 0.3044$).

## 3 Discussion

Based on the excitatory conditioning model of behavioral sensitization, conditioned locomotor activity and locomotor sensitization would be expected to concomitantly occur with different drug treatment regimens. However, in the present study, repeated morphine training did not generate the expression of locomotor sensitization after a 1-week withdrawal period but consistently led to the emergence of conditioned locomotion. Similar results have also been reported in other studies using different frequently abused drugs. Amphetamine and MDMA induced conditioned hyperlocomotion in the absence of sensitization [5,6]. By contrast, locomotor sensitization was observed in contexts that were not associated with drug administration and in the absence of any evidence of a CR [19,20]. These results indicated a dissociation between conditioned locomotion and contextual locomotor sensitization.

One possible reason for the observed effects is that locomotor activity may have been influenced by the emergence of stereotypy. Psychomotor stimulation induced by drugs typically involves increased locomotion at low doses and stereotyped behavior at high doses. With the increase in drug dose, the range of observed behaviors decreases, until animals exhibit stereotyped behaviors characterized by perseveration, including sniffing, licking, and chewing [21]. Increases in sniffing, licking, and chewing behaviors in a restricted area may suppress the expression of exploratory locomotion. Thus, it is necessary to verify whether or not this dissociation phenomenon between conditioned locomotion and context-dependent locomotor sensitization was confounded by the emergence of stereotyped behavior [22]. Nonetheless, in the present study, both locomotor activity and stereotypy in the challenge test showed consistent changes with different morphine treatment regimens. Therefore, the absence of locomotor sensitization was not attributable to the emergence of stereotyped behaviors. Another possibility is that the lack of a sensitization effect in the morphine (5 mg/kg)-paired group may have been attributable to a ceiling effect. The asymptote reached by the sensitized rats in the 3 mg/kg morphine-paired group was within the asymptote reached by animals that received an acute injection of 5 mg/kg morphine (locomotor activity: ~10000 counts over 4 h; stereotyped behavior: ~70 counts over 4 h). Our previous studies using a different experimental model found that these counts were >20000 over 4 h for locomotion and approximately 90 over 4 h for stereotypy with 5 mg/kg morphine pretreatment. Thus, the lack of sensitization effects in the morphine training groups was likely not attributable to a ceiling effect.

In summary, despite numerous behavioral pharmacology and neurobiology studies of sensitization, behavioral sensitization is not an inevitable consequence of the pharmacological actions of frequently abused drugs. The present results indicate that the expression of contextual sensitization depends on the interaction between treatment conditions, but the exact reasons for this remain unclear. Conditioned

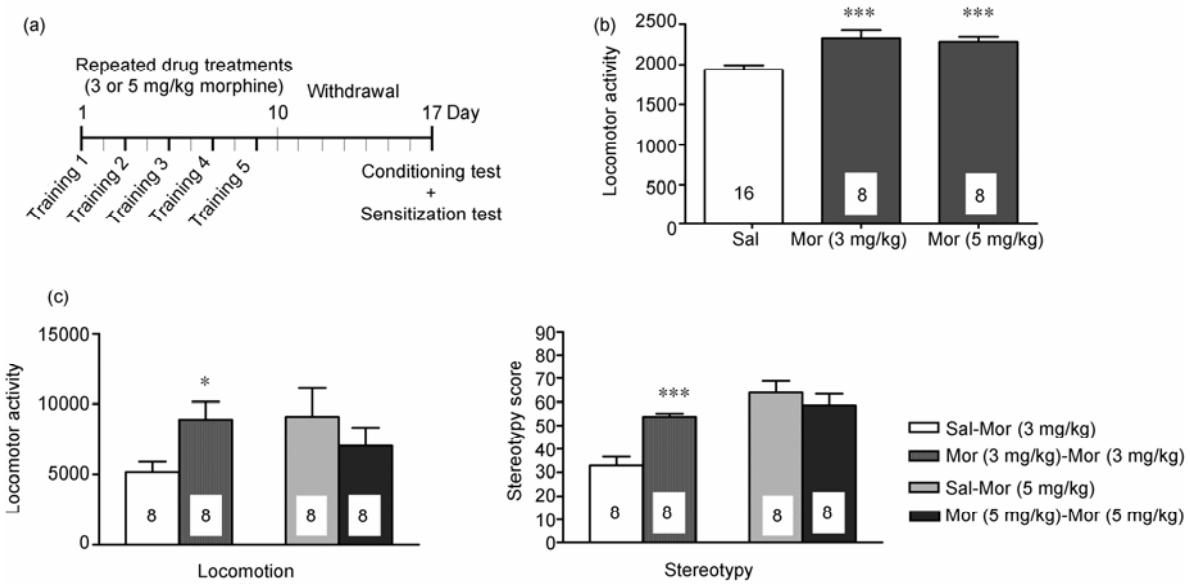


**Figure 2** Conditioned response and expression of sensitized behaviors in rats with no pre-exposure to the test environment during training. (a) Experimental schedule. (b) Conditioned locomotion. ***$P$<0.001, compared with saline training group (one-way ANOVA followed by Newman-Keuls *post hoc* test). (c) Contextual sensitized behaviors challenged by morphine, including locomotor activity and stereotypy. *$P$<0.05, ***$P$<0.001, compared with corresponding saline training group (Student's $t$-test).

hyperlocomotor activity induced by drug-related stimuli may reflect an "emotional state" of the animals related to the expectation of a drug reward.

## 4 Conclusion

The present study provides evidence that the expression of contextual sensitization is typically weaker and more susceptible to disruption than the CR, and a CR independently occurs from the drug dose regimen and environmental novelty. The present results suggest that different mechanisms may underlie these 2 behaviors and represent certain aspects of motivation-like behaviors associated with drug- related contexts.

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