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Delayed extinction and stronger drug-primed reinstatement of methamphetamine seeking in rats prenatally exposed to morphine

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ABSTRACT

Prenatal morphine (PM) affects the development of brain reward system and cognitive function. The present study aimed to determine whether PM exposure increases the vulnerability to MA addiction. Pregnant Sprague-Dawley rats were administered saline or morphine during embryonic days 3–20. The acquisition, extinction and reinstatement of methamphetamine (MA) conditioned place preference (CPP) and intravenous self-administration (SA) paradigms were assessed in the male adult offspring. There was no difference in the acquisition and expression of MA CPP between saline- and PM-exposed rats, whereas PM-exposed rats exhibited slower extinction and greater MA priming-induced reinstatement of drug-seeking behavior than controls. Similarly, MA SA under progressive ratio and fixed ratio schedules was not affected by PM exposure, but PM-exposed rats required more extinction sessions to reach the extinction criteria and displayed more severe MA priming-, but not cue-induced, reinstatement. Such alterations in extinction and reinstatement were not present when PM-exposed rats were tested in an equivalent paradigm assessing operant responding for food pellets. Our results demonstrate that PM exposure did not affect the association memory formation during acquisition of MA CPP or SA, but impaired extinction learning and increased MA-primed reinstatement in both tasks. These findings suggest that the offspring of women using morphine or heroin during pregnancy might predict persistent MA seeking during extinction and enhanced propensity to MA relapse although they might not be more susceptible to the reinforcing effect of MA during initiation of drug use.

1. Introduction

Substance abuse during pregnancy is a serious and growing problem. Children born to heroin- or morphine-addicted mothers suffer from high mortality and central nervous system impairments (Ostrea & Ostrea, 1997; Yanai et al., 2003) and present long-term neuropsychological consequences associated with dysfunction in intellectual ability, lack of emotional control, and disturbances in memory (Ornoy, 2003). Similarly, animals prenatally exposed to morphine showed spatial learning deficits in the Morris water maze and both working and reference memory impairments in the radial arm maze (Gass, Osborne, Watson, Brown, & Olive, 2009; Yang et al., 2003).

In general, the children of addicts are more likely than the general population to develop an addiction to drugs. Although many risk factors may be involved, gestational morphine exposure seems to be one of risk factors which lead to the offspring more prone to drug addiction. Preclinical studies have shown that prenatal morphine (PM) exposure increases vulnerability to morphine-induced conditioned place preference (CPP) and behavioral sensitization (Gagin, Kook, Cohen, & Shavit, 1997; Wu, Chen, Tao, & Huang, 2009). Moreover, the enhanced cocaine or heroin self-administration (SA) has been observed in the adult offspring prenatally exposed to morphine (Ramsey, Niesink, & Van Ree, 1993). These findings suggest that PM exposure induces a long-lasting enhancement of the reinforcing effects of morphine and cocaine.

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However, it remains unclear if PM exposure affects reinforcing effects of other abused drugs.

Methamphetamine (MA) is a commonly abused illicit drug, releasing excess dopamine into the synaptic clefts of dopaminergic neurons (Volz, Fleckenstein, & Hanson, 2007). As an extremely powerful and addictive psychostimulant, animal models of MA addiction including behavioral sensitization, CPP and SA have been well established (Gass et al., 2009; Rogers, De Santis, & See, 2008; Tien, Ho, Loh, & Ma, 2007). The present study aimed to determine whether PM affects MA CPP and SA because these two preclinical tasks were commonly used to compare the individual vulnerability to drug addiction (Tzschentke, 2007). The progressive ratio (PR) schedule in SA was used to investigate the potential effect of PM exposure on the motivation for MA in the present study. The PR schedule is an effective tool for studying the reinforcing efficacy of abused substances. The final ratio completed is defined as the breaking point, reflecting the maximum effort that an animal will expend in order to receive a defined drug infusion (Richardson & Roberts, 1996).

Furthermore, the extinction–reinstatement procedures of these two tasks suitable to study drug craving and relapse (Gass et al., 2009; Mueller & Stewart, 2000; Shaham, Shalev, Lu, De Wit, & Stewart, 2003) were included. Extinction is referred to the reduced responding when the conditioned stimulus or the reinforcer is no longer present (Bossert, Marchant, Calu, & Shaham, 2013). After successful acquisition of MA CPP, the animals underwent the non-confined extinction, in which the gradual reduction of the time spent in initially preferred compartment when the drug reward was absent. Following extinction, CPP was reinstated with a priming injection of a lower dose of MA. In the MA SA task, the drug seeking behaviors were reinstated by the conditioned cue and drug priming infusion after the extinction training reached the criteria.

Finally, the acquisition, extinction and reinstatement phases in an equivalent paradigm assessing operant responding for food SA were examined to reveal if PM exposure produced the same effects on operant conditioning of natural reinforcers.

2. Materials and methods

2.1. Animals

Pregnant Sprague-Dawley rats (BioLASCO Taiwan Co., Ltd) and their male offspring were used in the experiments. The pregnant female rats (at E2), 10–12 weeks old and weighing 200–250 g, were shipped from animal breeding company. After arrival, the dams were acclimatized to a room with controlled temperature (25 °C), humidity (50 ± 10%) and a 12 h day-night cycle (light on 07:00–19:00 h) for 24 h before experimentation. Rat dams during gestation and nursing were kept individually in separate cages and their offspring were housed 2–3 per cage after weaning. All animals were provided with food (Western Lab 7001, Orange, CA, USA) and water *ad libitum*. All procedure for animal care was proved by the Institutional Animal Care and Use Committee of the National Health Research Institutes.

2.2. Chemicals

Morphine and methamphetamine hydrochloride were purchased from the Taiwan Food and Drug Administration, Taipei, Taiwan. Morphine was dissolved in distilled water and methamphetamine hydrochloride was dissolved in physiological saline (0.9% NaCl).

2.3. Prenatal morphine exposure

Pregnant female rats (at embryonic day 2, E2) were randomly assigned into the control and morphine groups and received vehicle or morphine (s.c.) during E3–E20. The control group received distilled water 1 ml/kg, *s.c.*, twice a day. The morphine group received morphine, 2 mg/kg (initial dose) to 4 mg/kg (final dose), *s.c.*, twice a day (increment of 1 mg/kg per week). All rats received drug injections during 8:30–9:00 and 16:30–17:00. The dosage was selected to produce overt toxicity, but not overdose deaths (Chiang, Hung, Lee, Yan, & Ho, 2010).

2.4. MA CPP

The apparatus and procedure were described as a previous report (Kuo, Chai, & Chen, 2011). Briefly, CPP apparatus consisted of a large box made of wood and was divided into two large compartments of equal size (45 × 45 × 30 cm) by a wooden partition. One end compartment was painted gray and the other was painted with black and white vertical stripes on the walls. An unpainted small compartment was (36 × 18 × 20 cm), protruding from the rear of the two large boxes, connected the two entrances to allow animals move freely in the all three compartments. The apparatus was situated in a brightly lit room about 60 cm from a one-way vision window, preventing the rats from seeing any of the cues in the room.

The CPP procedures consisted of five consecutive phases, pre-exposure, conditioning, test, extinction and MA priming-induced reinstatement. On the first day of the experiment, animals were placed in the small compartment and allowed to explore the all three compartments for 10 min. In this pre-exposure phase, animals were allowed to habituate to the whole apparatus and their possible unconditioned place preference for compartments was verified.

Next, the conditioning phase consisted of 4 daily sessions and each rat was injected with MA (2 mg/kg, i.p.) and saline on the alternate days. Animals were placed into the specific compartment immediately after the injections. CPP test was conducted 24 h after the last conditioning session and no drug infusion was given before the test. Animals were allowed to move freely in the whole apparatus for 20 min as the pre-exposure and the time they spent in each compartment was recorded for CPP test analysis.

Extinction and MA-primed reinstatement were conducted as the CPP test. Extinction included 4 daily sessions. The amount of time the animals lingered in each compartment was recorded. The MA priming-induced reinstatement was manipulated 24 h after the last extinction session and MA (1 mg/kg, i.p.) priming injection was given 30 min before placing animals into the small compartment.

2.5. MA self-administration procedures

2.5.1. Food pretraining

Rats were food-restricted (5 g/day) for 48 h prior to starting food training. After the initiation of food training, animals received 12 g rat chow per day, at least 30 min after the end of the food training session. During the 1 h training session, the animals were trained to press the lever for a single food pellet (45 mg; Bioserve) under fixed ratio 1 (FR1). Only one lever was extended into the operant testing chamber during the initial food training period. Animals took 3–4 days to meet the criteria (defined as earning 100 food pellets within the 1 h session for three consecutive days).

2.5.2. Intravenous catheterization surgery

The IV catheterization surgery was conducted at least 3 days after the free feeding. Animals were anesthetized with isoflurane

(2% v/v). The external jugular vein was implanted with a Silastic tubing (ID = 0.51 mm; OD = 0.94 mm; Dow Corning Silastic) and the other end of the tubing was connected to an injection port of a harness (Instech, Plymouth Meeting, PA). The catheters were flushed daily with mix solution of baytril (2.5%; Bayer, Leverkusen, Germany) and heparinized saline (50 IU/ml) to preserve catheter patency. Animals were allowed to recover from the surgery for 5–6 days.

2.5.3. MA IVSA

The timeline of operant schedules for MA SA is shown in Fig. 2A. After recovery from surgery, rats were placed to the operant chambers (32 × 25 × 34 cm, Med associates Inc.) where the house light located on the wall opposite to lever was turned on. The house light was turned off to signal the starting of each session. The animals were allowed to self-administer MA under FR1 time-out 20 s (TO20 s) one lever schedule for 2 h during 4 consecutive days. The responding on the lever resulted in the delivery of the MA solution in a volume of 0.1 ml infused over 4 s (0.1 mg/kg/infusion; syringe pump model PHM-100, Med associates Inc.). A cue light, located above the lever, was activated simultaneously with the initiation of the MA infusion and it remained illuminated throughout the 20 s time-out period, during which responding was recorded but not reinforced. On day 5, a 3 h PR TO20 s schedule was conducted. The number of lever presses required to gain an infusion was determined by: $5 \times e^{(\text{infusion number} \times 0.2)} - 5$ (i.e., 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, etc.) (Richardson & Roberts, 1996). The PR schedule was terminated automatically if animals did not gain another infusion within an hour. The breaking point was defined as the final ratio completed by each animal.

Seventy-two hours after completing PR schedule, both groups were retrained to self-administer MA (0.1 mg/kg/per infusion) in 2 h daily sessions under FR1 TO20 s two-lever schedule. Unlike the FR1 schedule used above, two levers were inserted into the chamber. The successful responses on the active lever leaded to programmed reinforcement contingency, but any lever-response during the time-out or on the inactive lever did not have any consequences. After 2 daily FR1 TO 20 s sessions, the schedule was shifted to FR2 TO 20 s (3 days) and then, FR5 TO20 s (5 days). Once responding on the FR5 schedule was stable, animals were subjected to the daily 2 h extinction session, whereby pressing on the active lever no longer produced any programmed reinforcement consequence, namely no cue light presentation, nor the activation of the syringe pump. Extinction criteria were set when the subjects performed with the number of active lever presses 20% less than that of final 2 FR5 sessions in each group. Subsequently, animals underwent the MA priming- and cue-induced reinstatement tests (2 h each) using a within subjects design. A second extinction session was conducted between reinstatement tests (control: 3 days; PM: 4 days). For the MA-primed reinstatement, a MA (0.5 mg/kg, i.p.) infusion was given 30 min before the 2 h session. Responses on the active lever were recorded, but had no further presentations of a cue light or drug infusions. In the cue-induced restatement, the cue light ware presented after the successful active lever-press responses and this illumination continued through the TO period; however, no MA infusions were presented.

2.5.4. Food self-administration

The effects of PM exposure on food SA were assessed using a two-lever paradigm in separate groups of animals ($n = 6$ per group). The procedures were similar to that of the MA SA except that a single food pellet (45 mg; Bioserve) was delivered as the reinforcer. Animals took 3–5 days for acquiring lever press for food reinforcer on FR1 one lever schedule as determined by the daily gain of 100 food reinforcers for three consecutive days. Afterward, the responses were maintained in the two lever schedules shifted progressively in an order of FR1 TO20 s (2 days), FR2 TO20 s (3 days) and FR5 TO 20 s (5 days). The successful responses on the active lever produced the programmed consequence (e.g., a single 45-mg food pellet; 4 s representation of a cue light and 20 s time-out period). With a stable performance on FR5 schedule, animals were subjected to the 30 min daily extinction sessions, whereby presses on the active lever no longer produced any reinforcement contingency (i.e. no cue light presentation and no activation of the food dispenser). Similar to that of the MA SA experiment described above, the cue- or food-induced reinstatement test was manipulated when the reduction of operant responses reached the criterion (<20% of the final 2 FR5 sessions in each group).

Following extinctions, animals underwent the cue- and food priming-induced reinstatement. Responses on the active lever during cue-induced reinstatement test (30 min) produced a 4 s representation of a cue light and 20 s time-out period, but no food pellets were delivered. A second extinction session (2 days for each group) was manipulated between reinstatement tests. In the food-reinstatement test, a non-contingent presentation of two 45-mg food pellets was placed in the food dispenser immediately before the starting of food-induced reinstatement test. During the 30 min session, the food pellets were delivered for the first 15 successful behavioral responses to induce reinstatement and no more food pellets thereafter. The successful active lever responses always produced the time-out period, but no presentation of a cue light.

2.6. Statistics

The CPP data of acquisition, extinction and reinstatement were analyzed by two-way mixed design analysis of variance (ANOVA). For the SA task, only the numbers of the active lever presses were used for analysis. In the PR schedule, the group differences in the lever presses and break point were analyzed by one-way ANOVA. Performances during the FR schedules with one lever or two levers and cue-, MA priming- or food-induced reinstatement were analyzed by two-way mixed design ANOVA. The number of days needed to meet criteria of extinction was analyzed by *t*-test. Significance level was set at $p < 0.05$. All *post hoc* comparisons were made using paired or unpaired *t*-tests with correction for family-wise error.

3. Results

3.1. Effects of prenatal exposure to morphine on the dams and offspring

The control and PM groups were not significantly different in the number of offspring per litter, sex ratio, body weight and fatality at birth of offspring examined on postnatal day 1 (data not shown) as previous reports (Chen et al., 2015; Chiang et al., 2010). These results suggested that PM exposure did not cause serious physical consequences in the offspring.

3.2. Effects of PM exposure on the acquisition, extinction, and reinstatement of MA CPP

Two-way mixed design ANOVA with prenatal treatment as the between-subject factor and compartment (MA- or saline-paired) as the within-subject factor was used across acquisition, extinction, and reinstatement. PM exposure did not affect the acquisition of MA CPP (Fig. 1A). A significant main effect of compartment ($F(1, 14) = 32.71$, $p < 0.001$) was shown. Subsequent analysis indicated

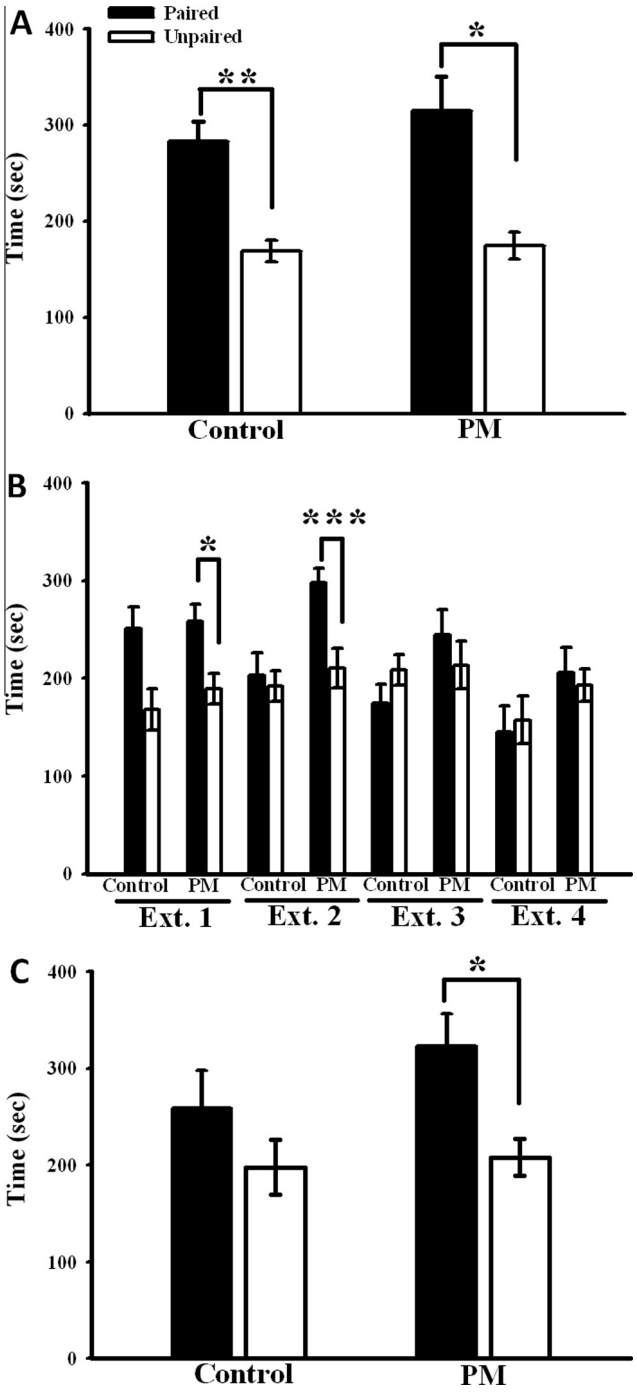

Fig. 1. Effects of PM exposure on MA-induced conditioned place preference. After conditioning, both control and PM groups showed the place preference for the MA (2 mg/kg)-paired compartment (A). PM group required more extinction trials to extinguish (B) and exhibited higher drug seeking behavior for MA after injection of a priming dose of MA (1 mg/kg, ip) than control group (C). Data were expressed as mean time (±SEM) in each compartment during the 20-min CPP acquisition, extinction and reinstatement tests ($n = 8$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with saline-paired compartment.

that both control and PM groups preferred MA-paired compartment (control: $t\,(7) = 5.16$, $p = 0.0013$; PM: $t\,(7) = 3.63$, $p = 0.0083$).

After CPP test, non-confined extinction was conducted by exposing animals to the apparatus without the drug treatments (Fig. 1B). On extinction day 1, a significant main effect of compartment ($F\,(1, 14) = 13.30$, $p < 0.01$), but not treatment or interaction, was revealed. *Post hoc* test demonstrated that only PM groups showed significant preference for MA-paired compartment, $t\,(7) = 3.09$, $p = 0.017$. On extinction day 2, significant main effects of compartment ($F\,(1, 14) = 11.37$, $p < 0.01$) and interaction ($F\,(1, 14) = 6.90$, $p = 0.019$) were shown. PM group showed preference for MA-paired compartment ($F\,(1, 14) = 17.99$, $p < 0.001$), whereas control group did not. There was no significant effect on extinction day 3–4.

MA priming-induced reinstatement was presented in Fig. 1C. There was a significant main effect of the compartment ($F\,(1, 14) = 11.97$, $p < 0.01$). Subsequent analysis revealed that significant difference between paired and unpaired compartment only shown in PM group ($t\,(7) = 2.89$, $p = 0.023$), but not in control group.

These results suggest that PM exposure did not interfere with CPP acquisition, but delayed the extinction and enhanced reinstatement.

3.3. Effects of PM exposure on a single-lever MA SA under fixed ratio 1 (FR1) and progressive ratio (PR) schedules

During the initial FR1 one lever training, the control and PM did not show differences in the lever presses (Fig. 2B) or drug infusions (data not shown). A PR schedule was used to assess the motivation to obtain MA in the control and PM groups. The data collected from operant responding on PR schedule show that the control and PM-exposed rats performed similarly. No significant difference in the lever presses (Fig. 2C) and the breaking point (Fig. 2D) between two groups was revealed. These data indicate that the acquisition of SA of MA in a single-lever operant chamber was not affected by PM exposure, nor was the motivation to earn this drug infusion.

3.4. Effects of PM exposure on the acquisition and extinction of MA SA using a two-lever paradigm

Two-way mixed design ANOVA with prenatal treatment as the between-subject factor and the training day as the within-subject factor did not yield a significant group difference under FR1, FR2, and FR5 schedules (Fig. 3A). The number of active lever pressing for the control and PM groups in the last FR5 session was 76.2 ± 15.04 and 67.33 ± 6.72, respectively. It appears that PM exposure did not affect acquisition of MA SA.

Two-way mixed design ANOVA with prenatal treatment as the between-subject factor and extinction day 1–8 as the within-subject factor showed the significant main effect (prenatal treatment: $F\,(1, 9) = 11.59$, $p < 0.01$; days: $F\,(7, 63) = 20.14$, $p < 0.001$), but not interaction. The last day for all the subjects in control and PM groups to meet the extinction criteria was day 8 and 13, respectively. The numbers of sessions for control and PM groups to reach the extinction criteria were 7.4 ± 0.6 and 11 ± 1.18, respectively. PM groups needed more days to meet the criteria of extinction than the control group ($t\,(9) = 2.92$, $p = 0.017$). Overall, PM-exposed rats displayed slower extinction than controls (Fig. 3B).

3.5. Effects of PM exposure on MA priming- or cue-induced restatement in MA SA

After extinction sessions, MA priming-induced reinstatement was examined (Fig. 4A). Two-way mixed design ANOVA with prenatal treatment as the between-subject factor and MA priming as the within subject factor revealed the significant effects of prenatal treatment, MA priming and interaction between prenatal treatment and MA priming (prenatal treatment: $F\,(1, 9) = 10.09$, $p < 0.05$; MA priming: $F\,(1, 9) = 24.66$, $p < 0.001$; Interaction: $F\,(1, 9) = 4.86$, $p = 0.054$). *Post hoc* tests demonstrated that PM group

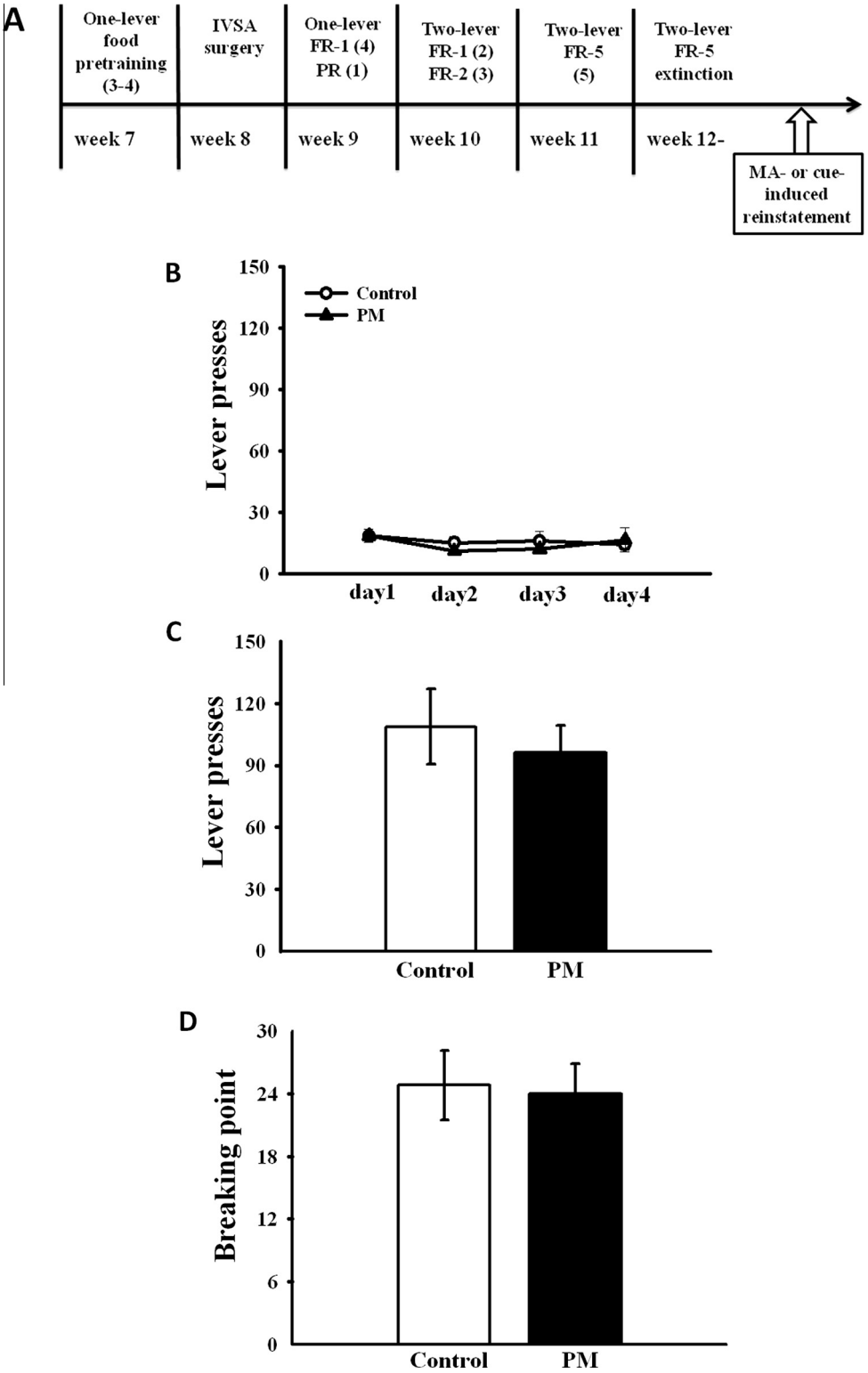

Fig. 2. Effects of PM exposure on one-lever MA self-administration paradigm under FR1 and PR schedules. Timeline of the MA SA and the numbers in parentheses represented the number of days of each operant schedule (A). PM group ($n = 6$) did not perform differently from control group ($n = 5$) under FR1 TO 20 s in 2 h daily sessions for 4 days (B). Lever-press responses (C) and break points (D) under a 3 h PR schedule were not significantly different between control or PM group. Data were expressed as mean ± SEM.

produced more responding than control group (t (9) = −2.82, $p = 0.02$) in the MA-primed restatement of drug-seeking behaviors. The animals underwent extinction training again (control: 3 days; PM: 4 days) prior to cue-induced reinstatement. Two-way mixed design ANOVA demonstrated significant main effect of cue (F (1, 9) = 7.85, $p < 0.05$) (Fig. 4B). However, *post hoc* tests did not reveal the group difference. These data indicated that MA drug-seeking behaviors were successfully reinstated by a MA-priming infusion and the conditioned cue. Furthermore, PM exposure exacerbated MA priming-, but not cue-induced reinstatement.

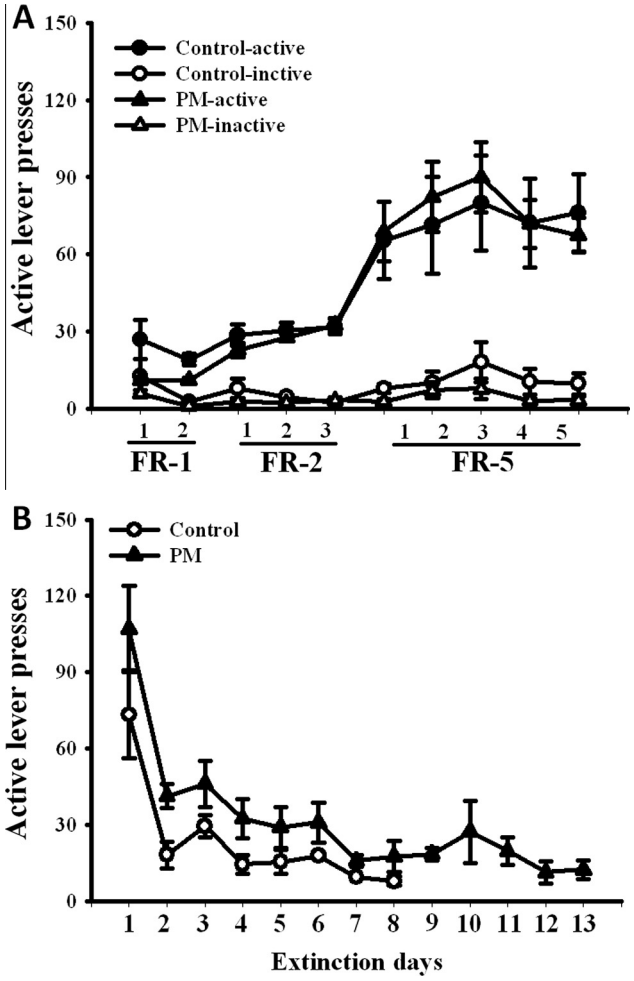

Fig. 3. Effects of PM exposure on acquisition and extinction of MA self-administration using a two-lever FR paradigm. The training curves of the control ($n = 5$) and PM groups ($n = 6$) were similar under the FR1 (2-day), FR2 (3-day) and FR5 schedules (5-day) during 2 h daily sessions (A). PM-exposed rats took more sessions to extinguish their operant responses than controls (B). Data were expressed as mean ± SEM.

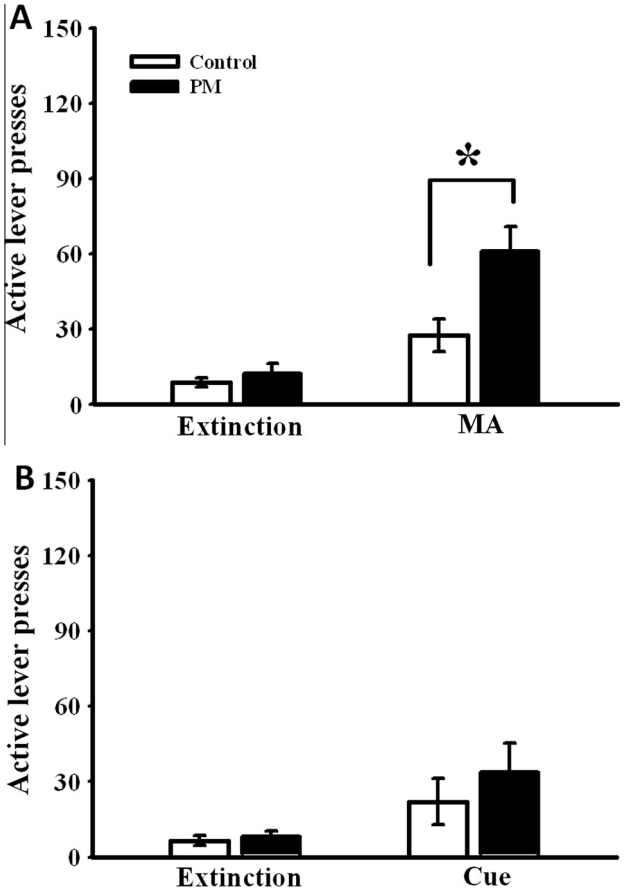

Fig. 4. Effects of PM exposure on the MA priming- or cue-induced restatement. PM group significantly increased their active lever presses than control group when the MA (0.5 mg/kg) priming injection was given 30 min before the test (A). The control and PM groups performed similarly in the cue-induced reinstatement (B). Data were expressed as the mean ± SEM (control: $n = 5$; PM: $n = 6$). $^{*}p < 0.05$ compared with control group.

3.6. Effects of PM exposure on the operant conditioning, extinction, and reinstatement of food SA

Two-way mixed design ANOVA with prenatal treatment as the between-subject factor and the training day as the within-subject factor revealed that the performance of the control and PM groups was not significantly different under FR1, FR2, and FR5 schedules (Fig. 5B). The lever-press responses were 737.33 ± 86.31 and 607.33 ± 114.56 for the control and PM groups in the last FR5 session. For the extinction, two-way mixed design ANOVA with prenatal treatment as the between-subject factor and extinction day as the within-subject factor showed the significant main effect of day (day: $F(2, 20) = 29.44$, $p < 0.001$), but not prenatal treatment or interaction. The extinction sessions needed to met the criteria were not significant different between control (4 ± 0.26) and PM (4.67 ± 0.40) group (Fig. 5C). Two-way mixed design ANOVA revealed a significant main effect of cue ($F(1, 10) = 5.94$, $p < 0.05$) in the cue-induced reinstatement (Fig. 5D) and food ($F(1, 10) = 38.24$, $p < 0.001$) in food-induced reinstatement (Fig. 5E). *Post hoc* comparisons did not show significant group difference. These results revealed that PM exposure did not affect the acquisition, extinction and both cue- and food-induced reinstatement of food SA.

4. Discussion

The present study examined the effects of PM exposure on the acquisition, extinction, and reinstatement of MA CPP and SA tasks. In addition, the motivation for MA using a PR schedule in MA SA was assessed. Control and PM-exposed rats did not show differences in the expression of MA CPP and MA SA and performed similarly in the PR schedule in the MA SA. However, PM-exposed rats took more sessions to extinguish their CPP and SA lever-pressing behaviors and displayed greater MA priming-induced reinstatement than controls in these two tasks. These results indicate that PM exposure impaired the putative inhibitory learning associated to extinction process that could increase the vulnerability of MA seeking behavior; conversely, PM exposure did not affect the initial memory formation involved in acquisition of drug conditioning and the motivation for MA. In contrast to those measured in MA SA, the acquisition, extinction, and both cue- and food-primed reinstatement of food SA were not affected by PM exposure. The present data support a notion that PM exposure specifically interferes with the extinction and reinstatement of MA seeking behavior, but leaves the natural reward, such as food, unaffected.

Our results are consistent with previous report that PM exposure did not affect MA-induced CPP (Chiang, Hung, & Ho, 2014). Similar manifestations were observed in MA SA. In fact, mid-to late gestational morphine exposure also did not alter morphine-induced CPP and SA (Riley & Vathy, 2006) and cocaine SA (Vathy,

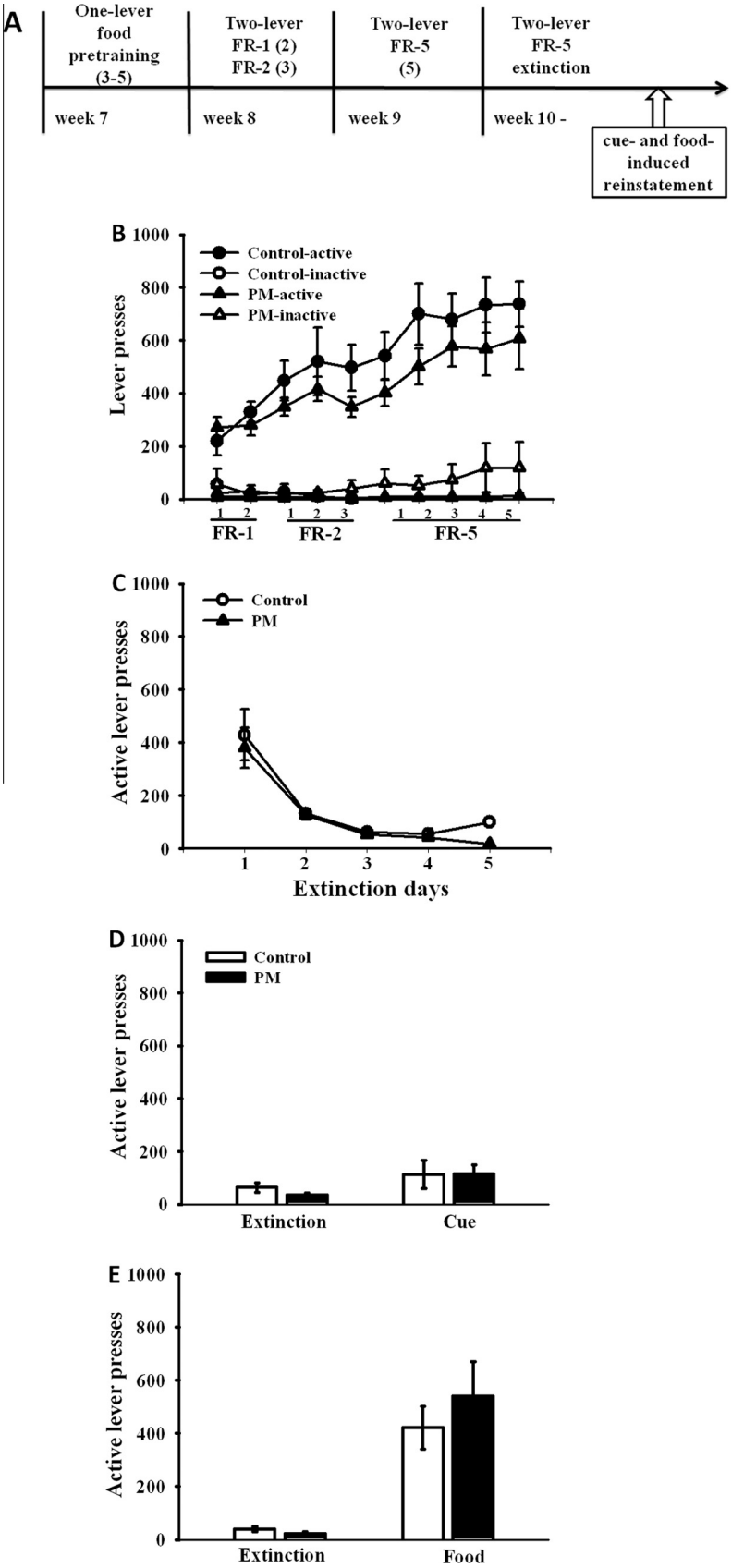

Fig. 5. Consequences of PM exposure on the operant conditioning, extinction and reinstatement of food self-administration. Timeline of the food SA and the numbers in parentheses represented the numbers of days of each FR schedule (A). The food pellets were given as the reinforcer under FR schedules (B) and animals were shifted to 2 h extinction sessions (C) after the stable FR5 performances. Cue- (D) and food-induced reinstatement (E) were followed. Data were expressed as mean ± SEM ($n = 6$). The numbers of subjects in each extinction day were 6 for day1–3, 5 for day 4 and 1 for day 5 in both groups.

Slamberova, & Liu, 2007). It appears that PM exposure did not alter the associative learning ability in classical and operant conditioning for MA, morphine, and cocaine. The results from MA SA under PR schedules showing no difference between control and PM-exposed rats further support that PM exposure did not influence the sensitivity to reinforcing properties of MA. Since only one dose of MA in CPP and SA was tested, further investigation is needed to reveal if PM exposure does not affect CPP and PR responses induced by higher or lower doses of MA.

One of the novel findings in the present study is that PM-exposed rats required more extinction sessions to extinguish MA CPP and SA operant response for MA. Extinction is defined as the gradual elimination of a learned response that occurs when the response is no longer reinforced or the unconditional stimulus is no longer presented in conjunction with the conditioned stimulus. In general, the delayed extinction in SA response is attributed to either a preservative responding of lever pressing or a slower rate of extinction learning. Since PM-exposed animals showed impaired extinction of MA CPP as well as MA SA, PM exposure might mainly affect the extinction learning rather than developing preservative responding.

It is noted that extinction creates a new inhibitory memory trace that is different from the original association memory (Rescorla, 2004). PM exposure did not affect the association memory formation during acquisition of MA CPP and SA, but altered the extinction learning in these two tasks. A recent report also demonstrated that PM exposure impaired the extinction of contextual fear extinction (Tan et al., 2015). It seems that extinction learning for drug and fear is sensitive to PM exposure. It is also of interest to reveal if PM exposure specifically influences extinction of drug-seeking behaviors for MA only or affects other abused drugs.

Slower rate of extinction for MA may reflect a stronger degree of original learning. However, the difference of the strength of the original learning between PM-exposed and control rats was not observed in the MA CPP and SA procedures used in the present study. Actually, our MA SA paradigm produced a gradual acquisition curve in which potentiated acquisition could be detected. Therefore, it is unlikely that extinction-related deficits in PM rats are attributable to increased levels of acquisition.

The possibility for slower rate of extinction learning might be explained by the incentive-sensitization theory of addiction (Robinson & Berridge, 2001; Robinson & Berridge, 2003; Vanderschuren & Pierce, 2010). In this perspective, repeated association of the rewarding effects of drugs and drug-related cues may induce greater motivational salience toward the cue, and thus are more difficult to extinguish. In our case, PM exposure may predispose the animals toward more hypersensitive ('sensitized') to MA and MA-associated contextual stimuli in CPP and SA tests, leading to the animals difficult to extinguish when exposed to MA-related context.

The sensitized motivation can also increase the risk of relapse. Reinstatement was induced by MA priming after the successful extinction in CPP and SA, and by conditioned-cue in SA as well. However, PM exposure enhanced the MA priming-induced reinstatement in CPP and SA, but left discrete conditioned cue (light)-induced reinstatement of MA seeking behavior in SA unaffected. The enhanced MA-primed, but not conditioned-cue reinstatement in SA was also shown in rats with long access of MA (Rogers et al., 2008). In contrast, the opioid receptor antagonist, naltrexone, attenuated MA-primed but not conditioned cue-induced reinstatement (Anggadiredja, Sakimura, Hiranita, & Yamamoto, 2004), implicating a potential role for the opioid system in MA-primed reinstatement. Studies on the neural substrates that maintain drug seeking have shown that the neural circuitries for different forms of reinstatement consist of partially overlapping yet distinctly different sets of brain nuclei (Feltenstein & See, 2008). PM exposure specifically enhanced MA-primed reinstatement, reflecting only the neural circuitry that mediates MA-primed reinstatement was affected (Feltenstein & See, 2008). It has been reported that PM exposure significantly increases the density of mu-opioid receptors in the nucleus accumbens (Vathy, Slamberova, Rimanoczy, Riley, & Bar, 2003), a critical brain region for drug-primed reinstatement. Whether this alteration is related to the stronger MA-primed reinstatement in PM-exposed rats remains to be determined.

It is of note that contextual cues and discrete conditioned cues are involved in SA task. Although contextual and discrete conditioned cues associated with drug treatments, conditioning to contextual cues versus discrete cues is mediated by different, albeit overlapping neural systems, one being hippocampal-dependent and one being hippocampal-independent (Phillips & LeDoux, 1992). It seems likely PM exposure specifically increased the strength of the learned association between MA-reward and the MA-related context, independent of MA-paired cue light. In fact, the impaired contextual fear extinction by PM exposure has been mirrored by abnormalities in synaptic plasticity in the Schaffer collateral-CA1 synapses of the hippocampus (Tan et al., 2015). Therefore, it is possible that PM exposure resulted in an exceptional sensitivity in hippocampal-dependent neural circuits involved in transference of MA-reward salience to the MA-related context, thus PM-exposed rats displayed delayed extinction and stronger MA-primed reinstatement in CPP and SA tests.

In contrast to the MA SA, the delayed extinction was not present when PM-exposed rats were tested in an equivalent paradigm assessing operant responding for food pellets. It is likely that the mechanisms underlying different types of appetitive extinction are distinct. Several studies have demonstrated that genetic manipulations altered drug-related extinction behavior, but did not affect the extinction of food or sucrose-seeking behavior (Briand, Lee, Blendy, & Pierce, 2012; Chesworth, Brown, Kim, & Lawrence, 2013). Therefore, although both MA and food are appetitive reinforcers, MA extinction may rely on mechanisms distinct from those engaged in food extinction. PM exposure specifically affects the neural circuits responsible for MA extinction, but leaves those for food extinction intact.

In summary, the present study demonstrated that PM exposure selectively delayed the extinction and enhanced drug priming-induced reinstatement of MA seeking behavior in both CPP and SA tasks. The results suggest that the offspring of women using morphine or heroin during pregnancy might predict persistent MA seeking during extinction and enhanced propensity to MA relapse, leading to increasing the difficulty in quitting and increased addiction severity. However, morphine-exposed offspring might not be more vulnerable to the reinforcing effect of MA during initiation of drug use because neither the training process to acquire MA CPP and MA SA, nor the reinforcing efficacy of MA on the PR schedule was affected by PM exposure. These findings provide a complete picture of the MA addiction propensity and severity after gestational morphine exposure.

Author contributions

Conceived and designed the experiments: HHC STC YLS PLT. Performed the experiments: YLS TYC TWH. Analyzed and interpreted the data: YLS TYC HHC. Contributed reagents/apparatus/materials: STC RML MHC PLT. Wrote the manuscript: YLS HHC MHC RML.

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