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Pharmacology, Biochemistry and Behavior 68 (2001) 389–394

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Spatial learning and morphine-rewarded place preference negatively correlates in mice

Nan-Jie Xu[a,b], Ling-Zhi Wang[a], Chun-Fu Wu[b], Gang Pei[a,b,*]

[a]*Shanghai Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yue Yang Road, Shanghai 200031, China*

[b]*Department of Pharmacology, Shenyang Pharmaceutical University, 103 Wen Hua Road, Shenyang 110015, China*

Received 27 March 2000; received in revised form 18 October 2000; accepted 2 November 2000

Abstract

Accumulating evidence has indicated that there might exist some correlation between opiate reward and certain kinds of learning and memory processes. The present study attempted to investigate the correlation between individual differences in morphine reward and capacities in spatial learning and spontaneous alternation. In the present studies, good-response (GR) and poor-response (PR) mice were respectively selected according to their performance in a spatial learning test involving the Morris water maze or in a spontaneous alternation task using the Y-maze. In a place preference conditioning procedure, morphine (3.0 mg/kg) produced significant conditioned place preference (CPP) in both GR and PR mice selected by using either the Morris water maze or the Y-maze. The PR mice selected with the Morris water maze showed significantly more CPP induced by morphine than the GR mice. However, no detectable difference was observed in morphine-induced CPP between the GR and PR mice selected with the Y-maze. These results suggested that the variation in morphine-induced CPP in mice is somehow differentially related to that of spatial learning but unlikely to that of spontaneous alternation.

Keywords: Morphine; Conditioned place preference; Morris water maze; Y-maze spontaneous alternation task

1. Introduction

Opiate addiction has been considered a complex phenomenon involving many biological and social factors, and it is also believed to involve individual differences in brain functions, behaviors, as well as genetic background. There is accumulating evidence suggesting that the rewarding process elicited by opiates may share a common mechanism with other types of neural plasticity such as learning and memory. For example, inhibition of some key processes involved in learning and memory, such as *N*-methyl-D-aspartate (NMDA) receptor (Li et al., 1997; Stecher et al., 1997), nitric oxide synthase (Zou et al., 1998), and cAMP response element binding protein (Guzowski and McGaugh, 1997; Lamprecht et al., 1997), effectively prevents the development of opiate tolerance and dependence (Del Poze et al., 1996; Lane Ladd et al., 1997; London et al., 1995; Trujillo and Akil, 1991). Our previous studies also showed that inhibition of brain muscarine receptor (Zhou et al., 1999) and hippocampal calcium/calmodulin-dependent protein kinase II (CaMKII) (Fan et al., 1999), both of which are essential in learning and memory, could attenuate the development of morphine tolerance and dependence. Furthermore, our experiments at the cell and molecular level indicated that NMDA receptors and CaMKII are involved in opioid receptor function and its signal transduction (Cai et al., 1997; Fan et al., 1997, 1998; Lou et al., 1999). These results suggest that there might exist some correlation between opiate reward and certain kinds of learning and memory processes.

It is well known that animals show significant individual differences both in performing learning and memory tasks and responding to opiate reward. However, it is not clear if the individual differences in learning and memory are intrinsically related to differential sensitivity to opiate reward. The present study revealed that spatial learning performance and morphine-rewarded place preference negatively correlates in mice.

* Corresponding author. Fax: +86-21-64718563.
E-mail address: gangpei@sunm.shcnc.ac.cn (G. Pei).

0091-3057/01/$ – see front matter
PII: S0091-3057(00)00479-2

2. Methods

2.1. Animals

Male outbred ICR mice, including a total of 380 mice for Morris water maze screening and of 355 mice for Y-maze screening, were obtained from Shanghai Center of Experimental Animals at 6 weeks before training, and housed in a temperature-controlled (22°C) colony room that was maintained on a standard 12-h light/12-h dark cycle with food and water available. Experiments were carried out between 10:00 a.m. and 6:00 p.m. in a soundproof laboratory, to which mice were habituated at least 30 min before each experiment. All animal treatments were strictly in accordance with the National Institutes of Health Guide of the Care and Use of Laboratory Animals.

2.2. Morris water maze

2.2.1. Apparatus

The Morris water maze (Morgan et al., 1998; Morris, 1984) consisted of a steel circular pool (115 cm in diameter, 68 cm in height) that was partially filled with water (24°C). Milk powder was used to render the water opaque. The pool was divided into four quadrants with four starting locations called north (N), east (E), south (S), and west (W) at equal distances on the rim. An invisible escape platform (10 cm in diameter) was submerged 1 cm below the surface and placed in the center of the northeast quadrant.

2.2.2. Procedure

The procedure was conducted using a minor modification of the method previously described (Morgan et al., 1998; Morris, 1984). During the training period of the task, mice were given four trials per day to find the hidden platform for 5 consecutive days. Each mouse was gently placed into the water with the nose pointing toward the wall at one of the starting points, which varied from trial to trial, and the latency to find the platform was recorded up to 60 s. Mice were allowed to remain on the platform for 15 s, and then removed from the maze to its home cage. If the mouse did not find the platform within 60 s, the latency was assigned as 60 s, and the mouse was manually placed on the platform, left for 15 s, and returned to its original cage. The escape latency (EL), i.e., the time required for the mouse to climb onto the platform, was recorded. A probe trial, in which the escape platform was removed from the pool, was performed on Day 6, and the mouse was allowed to search for 60 s. The time spent in the trained quadrant, which had previously contained the hidden platform, was recorded. In the visible platform trial, which was carried out on the same day after the probe trial, to ensure the mice could perform the task, the platform was made apparent using a dark mat with white cross-hatched stripes and two small flags were placed on top of the platform to indicate the position in the pool. Mice were started in the pool in a similar manner as the hidden platform task and given four 60-s trials, during which the time to reach the platform was recorded. On Day 7, 24 h after the probe trial, the animals were tested for retention. The platform was submerged again and the EL was recorded. The mice were separated at two extreme ends according to their performance, and were designated as the good-response (GR) or the poor-response (PR) groups, both of which were confined to about 10% of the total animals. The mice with both an EL of <15 s on Day 5 and a searching time of >20 s spent in the trained quadrant on Day 6 were selected as GR mice, and those with both an EL of >45 s and a searching time of <18 s were categorized as PR mice. These mice were tested later in the conditioned place preference (CPP) test.

2.3. Spontaneous alternation performance

2.3.1. Apparatus

Spontaneous alternation performance was assessed in mice by using a symmetrical Y-maze as described previously (McNay and Gold, 1998; Morgan et al., 1998; Ragozzino et al., 1992; Sarter et al., 1988). The maze was made of black plastics. Each arm was 25 cm long, 15 cm high, 15 cm wide, and was randomly designated as A, B, or C.

2.3.2. Procedure

Mice were allowed to roam freely through the maze during an 8-min trial, and the series of arm entries, including possible returns into the same arm, were recorded. The number of arm entries made by the mouse was used to estimate activity. An alternation was defined as entries into all three arms on consecutive occasions (i.e., ABC, ACB, CAB, etc.). The number of maximum alternations was, therefore, the total number of arm entries minus two and the percentage of alternation was calculated as (actual alternations/maximum alternations) $\times 100$. For example, if the mouse performed ABCACBACCAB, the number of arm entries would be 11, and the successive alternations: ABC, BCA, ACB, CBA, BAC, CAB. Therefore, the percentage of alternation would be $[6/(11-2)] \times 100 = 66.7$. For spontaneous alternation performance, the GR and PR mice were also confined to about 10% of the total screened animals at two extreme ends. The GR was designated as performance with more than 75% alterations and the PR with less than 45%. These mice were also used later in the CPP test.

2.4. Conditioned place preference

2.4.1. Apparatus

The CPP apparatus consisted of two compartments (30 cm long $\times$ 15 cm wide $\times$ 15 cm high) separated by a guillotine door. One compartment had a white wall with

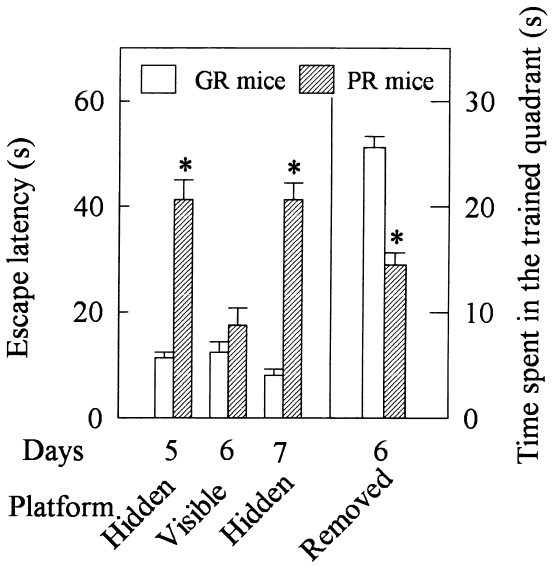

Fig. 1. Mice selection using the Morris water maze. The trials were carried out with the platform hidden (on Days 5 and 7), visible (on Day 6), and removed (on Day 6). The GR mice ($n=38$, with both an EL of <15 s and a time of >20 s spent in the trained quadrant) and the PR mice ($n=34$, with both an EL of >45 s and a time of <18 s spent in the trained quadrant) were selected from a total of 380 mice tested according to their performance. Results are expressed as mean ± S.E.M. $^*P<.001$ vs. the GR group.

black stripes and a floor with a textured surface, whereas the other had a black wall with white spots and a smooth floor.

2.4.2. Procedure

The CPP procedure was conducted using a minor modification of the method previously described (Del Poze et al., 1996; Suzuki et al., 1993), and consisted of three phases: (1) preconditioning phase, (2) conditioning phase, and (3) testing phase. During the preconditioning phase, the mice were adapted to the test apparatus for 5 min. During the conditioning phase of 4 consecutive days, the animals were conditioned with saline and morphine (3 mg/kg). For conditioning, mice were injected with morphine and immediately confined to one of two compartments. Following saline injection, they were immediately confined to the other compartment. Conditioning sessions (four drug and four saline sessions), each 30 min in duration, were conducted over 4 days. Mice received two trials daily with at least 4 h separating the drug and saline training sessions. The saline control groups for GR and PR mice were treated with saline in both sessions and consequently were saline-paired with both compartments. During the testing phase, the mouse was placed in a compartment with access to both chambers for 15 min. The time spent in each compartment during the 15-min test period was measured. The difference in the time spent at the drug- vs. saline-paired compartment was used as a measure of the degree of CPP. The number of shuttles between two sides in the testing phase was also recorded to estimate activity.

2.4.3. Drugs

Morphine hydrochloride was prepared at concentrations of 3 mg/kg in a saline solution and injected subcutaneously in a volume of 1 ml/kg body weight. Injection of saline (0.9% NaCl) were also in a volume of 1 ml/kg body weight.

2.5. Statistical analysis

Results are expressed as means ± S.E.M. The data from the Y-maze and the Morris water maze were analyzed by a two-tailed t test. For comparison of the data from the CPP test, a two-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test were performed. In all tests, the criterion for statistical significance was $P<.05$.

3. Results

Mice show significant individual differences in behavioral responses in the Morris water maze and the Y-maze, and could easily be separated at two extreme ends according to their performance, designated as the GR or PR groups in this study. As shown in Fig. 1, the GR mice showed a significantly shorter EL (Day 5, $t=7.22$, $P<.001$) and a considerably longer time (Day 6, $t=7.05$, $P<.001$) in the trained quadrant than the PR mice. However, on the visible platform trail on Day 6, no significant difference was found between the two groups ($t=1.11$, $P>.05$), indicating the similar capacities of vision and swimming. Furthermore, the differences reappeared in the succeeding hidden platform trial (Day 7, $t=10.25$, $P<.001$, Fig. 1). Similarly, mice could be classified into GR and PR groups according to their performance in spontaneous alternation task on the Y-maze (Fig. 2). The alternation percentage of GR mice was about twice that of the PR mice ($t=18.04$, $P<.001$), while

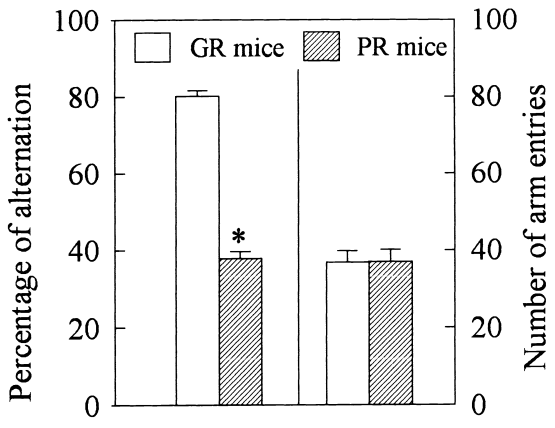

Fig. 2. Mice selection using the Y-maze spontaneous alternation task. The GR mice ($n=42$, with more than 75% alterations) and the PR mice ($n=30$, with less than 45% alterations) were selected from a total of 355 mice, and their activities were reflected by the number of arm entries. Results are expressed as mean ± S.E.M. $^*P<.001$ vs. the GR group.

they behaved similarly as reflected by the number of arm entries ($t=0.03$, $P>.05$).

In the CPP test, for the GR and PR mice from the Morris water maze test selection, a two-way AVOVA revealed significant difference between drug treatments [$F(1,68)=13.6$, $P<.001$], between groups [$F(1,68)=69.8$, $P<.001$], and the interaction between two factors [$F(1,68)=5.17$, $P<.05$], as shown in Fig. 3A. Subsequent post hoc comparisons revealed that both the GR and PR mice showed reliable CPP induced by morphine (both $P<.001$). Moreover, the PR mice spent about twice as much time on the morphine-paired side as the GR mice ($P<.001$, Fig. 3A), which was apparently not due to their lack of locomotor activity since no detectable effects of

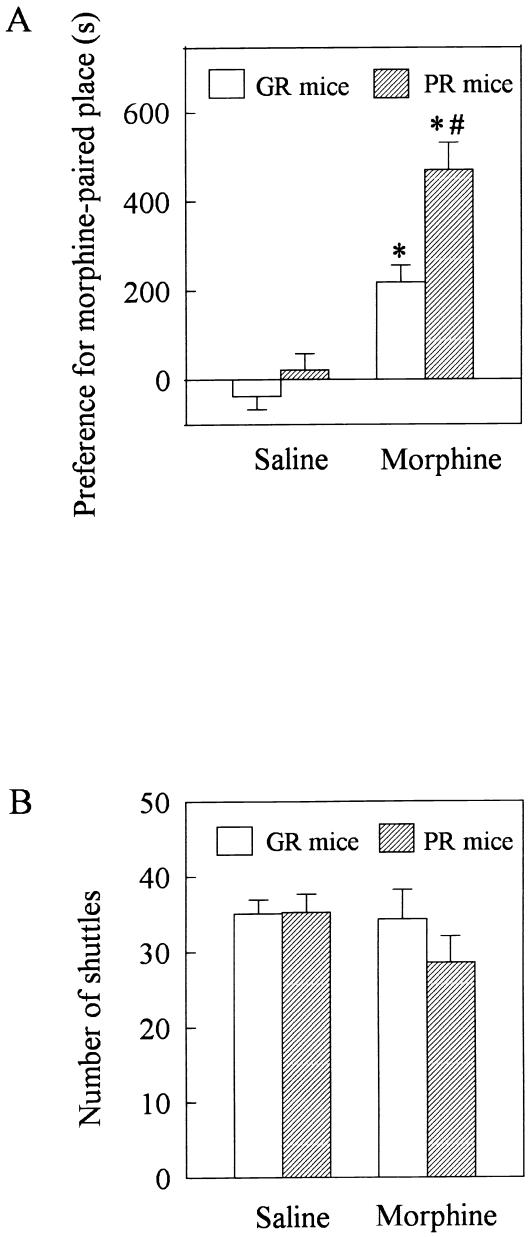

Fig. 3. (A) Morphine-induced CPP in the GR mice and the PR mice ($n=17-20$) selected from the Morris water maze task. The data were expressed as the mean difference between times spent on morphine- and saline-paired sides of the test box (mean ± S.E.M.). (B) The activity of mice was estimated by recording the number of shuttles between the two compartments during the testing phase. $^{*}P<.001$ vs. the saline groups, $^{\#}P<.001$ vs. the GR group.

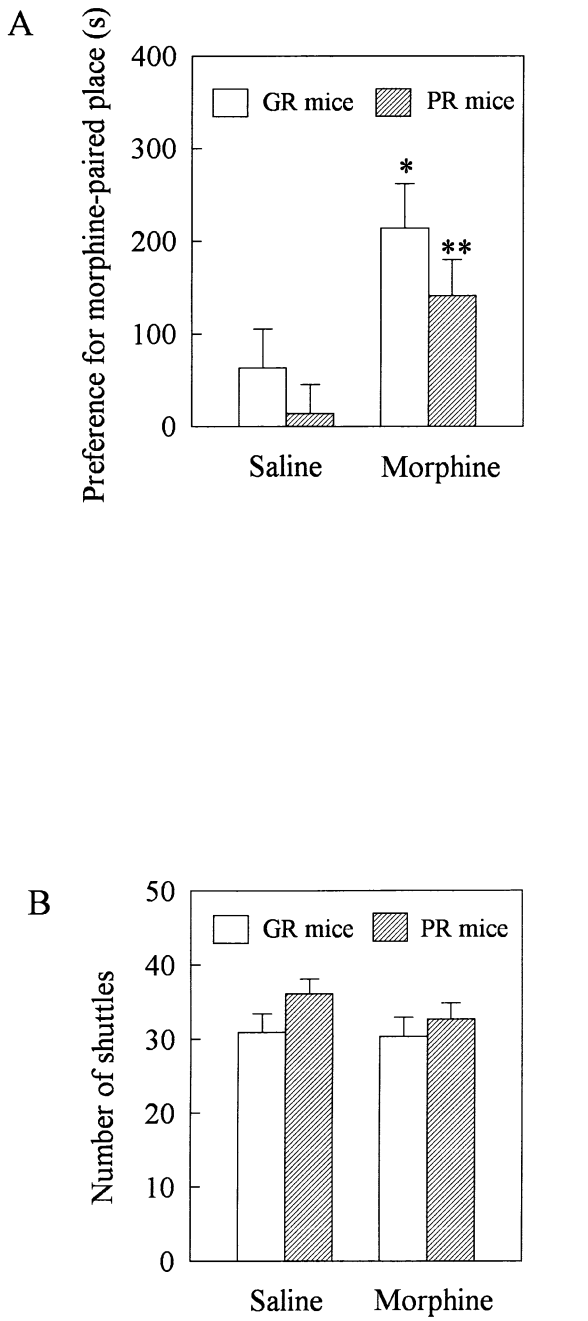

Fig. 4. (A) Morphine-induced CPP in the GR and the PR mice ($n=15-19$) selected from the Y-maze. The data were expressed as the mean difference between times spent on morphine- and saline-paired sides of the test box (mean ± S.E.M.). (B) The activity of mice was estimated by recording the number of shuttles between two compartments during the testing phase. $^{*}P<.05$, $^{**}P<.01$ vs. the saline groups, no significant differences were observed between the GR and the PR mice.

morphine [$F(1,68) = 0.86$, $P > .05$], groups [$F(1,68) = 1.51$, $P > .05$], or their interaction [$F(1,68) = 0.94$, $P > .05$] could be observed in the number of shuttles between the two groups (Fig. 3B).

Interestingly, as shown in Fig. 4, in contrast to the mice selected from the Morris water maze task, analysis revealed significant effects of morphine [$F(1,68) = 12.1$, $P < .001$], but no effect of groups [$F(1,68) = 2.35$, $P > .05$], or the interaction between two factors [$F(1,68) = 0.08$, $P > .05$] on CPP in the GR and PR mice selected from the Y-maze task.

4. Discussion

It is commonly known that notable individual variation exists in behavioral responses in animals. Many studies have been carried out to characterize such variation and this has been suggested to account for the individual differences in vulnerability for drug addiction (Deroche et al., 1993; Sills and Vaccarino, 1998). Our present experiment provides experimental evidence for the existence of reliable individual differences of animals in learning and memory ability, as tested by a spatial navigational learning test and a spontaneous alternation task. Thus, mice could readily be separated at two extreme ends according to their performance, designated as the GR or PR groups in this study. Although it is not clear what accounts for the individual differences in the behavioral responses, the differences between the GR and PR mice are unlikely to be due to individual variations in sensorimotor function, swimming ability, or locomotor activity, which have been excluded in this study.

Many factors, such as genetic background, neuronal development, environmental stimuli, and psychological motivation, could be involved in the above individual differences. If so, there might exist interconnections among the variation of the behavioral responses. The present study attempted to investigate the correlation between individual differences in morphine reward (CPP) and the capacities in spatial learning (Morris water maze) and spontaneous alternation (Y-maze). Our data demonstrate that the variation in morphine-induced CPP in mice is somehow differentially related to that of spatial learning, suggesting that some casual relationship may exit between the two behavioral measures. Interestingly, in contrast to the mice selected from the Morris water maze task, no difference was observed between the GR and PR mice selected from the Y-maze task, an index of spatial working memory (McNay and Gold, 1998; Sarter et al., 1988). The possible explanation for this apparent discrepancy is that not only the modality but also the complexity of the cue constellations are different between the two tasks. The Y-maze task primarily tests exploratory behavior and short-term (working) memory, and the Morris water maze task tests long-term memory. Furthermore, the Y-maze may be based on simple egocentric or intra-maze cues whereas the Morris water maze task can only be solved using extra-maze visual spatial cues (Morris, 1984). Our preliminary results (data not shown) revealed that the GR mice from the Morris water maze selection showed higher percentage of alternation than the PR mice when they were tested in the Y-maze, while the GR and PR mice selected from the Y-maze task performed indistinguishably on the Morris water maze task. Therefore, the spatial learning task and the Y-maze spontaneous alteration task do not appear to be equivalent screening methods.

There are many reasons why individual differences exist in morphine reward. One of these reasons has been tied to the intrinsic variation in the functioning of the mesolimbic dopamine system (Sills and Vaccarino, 1998). Deroche et al. (1993) also reported that individual vulnerability to morphine was predicted by reactivity to novelty as well as corticosterone response. Furthermore, there is strong evidence that individual differences in dopamine release, some signaling phosphoproteins, and genotype may contribute to individual differences in vulnerability for morphine reward (Beitner Johnson et al., 1991; Glick et al., 1992; Shoaib et al., 1995; Spanagel et al., 1996). However, the underlying mechanisms for the correlation between individual differences from the tests of CPP and Morris water maze remain unknown. One speculations for the correlation is that the strategy needed in the Morris water maze, which requires ability of accurate directionality to identifying the position of the platform by continual monitoring of the animal's position in relation to extra-maze cues (Morris, 1984), may be unfavorable for the mice to perform the morphine reinforced learning (CPP). On the other hand, the sensitivity of mice to stress, a possibly potential negative factor in Morris water maze (de Quervain et al., 1998), may also influence the PR mice, to some extent, to display place preference after morphine treatment. However, this still remains to be further investigated.

Acknowledgments

This work was supported by research grants from National Natural Science Foundation of China (39630130, 39625015, and 39825110), Chinese Academy of Sciences (KJ951-B1-608 and KY951-A1-301), and the German Max-Planck Society. The authors thank Professor Zhen-Tong Mei for helpful suggestions, and Su-Hui Duan, Guo-Bin Bao, Hui Zhou, Xin Ge, Lin Lu, and Ying Xong for their technical assistance.

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