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# Failure to Discriminate Conspecifics in Amygdaloid-Lesioned Mice[1]

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BORLONGAN, C. V. AND S. WATANABE. *Failure to discriminate conspecifics in amygdaloid-lesioned mice.* PHARMACOL BIOCHEM BEHAV **48**(3) 677–680, 1994.—An experiment was conducted to investigate the role of the amygdala in the discriminative behavior of mice using the conditioned individual preference (CIP) method, a modified conditioned place preference (CPP) paradigm. CIP training of the subject mice involved 6 consecutive days of alternate IP injections of morphine (3 mg/kg) or saline followed by associations with a stimulus mouse in one compartment of the preference box. After the CIP training, the subject mice were given a choice between the morphine-associated and the saline-associated stimulus mice. Normal and sham-operated mice showed preference for the morphine-associated stimulus mouse. On the other hand, mice receiving bilateral amygdala lesions before or after CIP training did not show any preference for either stimulus mouse. These results support the view that the amygdala influences expression and acquisition of conditioned discriminant behaviors of mice by possibly interacting centrally, through its opiate receptors, with the peripherally injected morphine.

Morphine Opiate receptors Amygdala Conspecific discrimination Conditioned place preference Conditioned individual preference Mice

THE occurrences of individual, sibling, mother-infant, colony and olfactory discriminations in mice were successfully observed using the conditioned individual preference (CIP) method (3–5). This CIP method is a modified conditioned place preference (CCP) paradigm. CPP tests typically use an experimental space with at least two discriminable places. One place is distinct by making its texture or color different from the other place. Here in CIP, however, we made both places homogenous except that we put a different stimulus mouse in each place. Following the basic principle of CPP, if the drug being tested has reinforcing properties, then after conditioning there would be a shift in the time spent in the drug-associated place, in our case the drug-associated stimulus mouse. CIP differs from CPP in that it investigates the animal's preferential behavior instead of the discriminative properties of the drug. We have observed that at a low dosage, morphine can establish individual preference (3–5). However, we have yet to investigate the brain mechanism involved in the observed conditioned preference. Because we have successfully shown the occurrence of preference using morphine, it is logical to look for the opioid system that exists in the brain. Several behavioral studies have successfully used opioids as agents of putative conditioning to establish a place preference (1,2, 6,11,14,16). Autoradiographic studies have shown that opiate receptors are highly concentrated in numerous structures of the limbic system, especially the amygdala [reviewed in (18)]. Recently, a lesion study revealed that damage to the amygdala impairs acquisition of the CPP task (13). We focused on the possible role of the central nucleus of the amygdala on the morphine-conditioned individual discrimination in mice.

## METHOD

### *Subjects*

We used 10 3-week-old male Crj ICR mice for each experiment. The mice were obtained from Charles River Japan Breeding Company. Immediately upon arrival, all subjects were individually housed in a cage made of transparent Plexiglas. The cages were placed in one room with temperature set at 23 ± 2°C and under a 12 L : 12 D cycle. Food and water were freely available.

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*Stimuli*

For each subject, we used a pair of 3-week-old male Crj ICR mice also obtained from the same breeding company. The stimulus mice later associated with morphine were separately housed from those mice paired with saline. All stimuli mice were housed with the subjects in the same room. Food and water were also freely available.

*Apparatus*

Details of the preference box have been described previously (3). Briefly, the box (32 × 22 × 14 cm) was divided into two compartments. Two small stainless steel cages (4.5 × 8 × 9 cm) were individually attached, fronting one another, on the walls of each compartment. A stimulus mouse was placed in each cage during experimental sessions.

*Behavioral Procedure*

Details of the conditioning procedure have been described previously (3). In group 1, each normal subject mouse was first habituated to the preference box together with a pair of stimulus mice for 15 min daily for 2 consecutive days. During the second habituation day, the time spent by the subject mouse staying in either compartment was recorded. Conditioning then followed with 6 alternate treatment days of morphine (3 mg/kg, IP, purchased from Sankyo Company) and saline. Immediately after each injection, the subject mouse was placed with one stimulus for 60 min in one compartment of the box. Plexiglas was inserted in the middle of the box to restrict movement of the subject to the one compartment that contained the stimulus. On the day following the last conditioning session, tests were conducted using the previous pair of stimulus mice. The time spent in each compartment was recorded as in the habituation phase. In group 2, prior to conditioning, 10 subject mice underwent bilateral amygdaloid lesioning with coordinates: anterior = −1.0 mm; lateral = ±2.6 mm; ventral = −4.6 mm (17). The subject mouse was first anaesthetized with 10 mg/kg of ketamine (Sankyo Company) and held in a stereotaxic apparatus. Lesions were made through a radio frequency lesion generator system (Radionics RFG 4A model), with currents being passed through an electrode (0.3 mm). The electrode tip temperature was maintained at 75–85°7C for 2 min. A recovery period of 5–7 days was allowed prior to conditioning. In group 3, another group of 10 mice underwent the same surgical procedures as in group 2 but lesions were conducted on the day following the last conditioning session. Again, the same recovery period was allowed prior to testing. In group 4, another group of 10 mice were subjected to the surgical procedures but lesions were not generated.

*Histology*

The subjects in the last three experiments were later sacrificed by transcardial perfusion with isotonic saline and 4% formalin in a 0.9% NaCl solution with pH 7.4. The brains were removed and fixed for at least a day in the perfusion medium, and then cut in a microvibrotome (DSK Microslicer, DTK 1000) at 50 μm. Every section was saved and stained with cresyl violet and luxol tase blue according to the method of Kluver and Barrera (9). The sections were inspected under an electron microscope, and drawings were made from the microscopic projections. The selection of acceptable lesions was made independently by two different persons.

RESULTS

Histological results revealed that all animals receiving lesions had acceptable symmetrical lesions concentrated at the region of the central nucleus of the amygdala. Figure 1 shows an example of a brain of a mouse that had received amygdala lesions. In all animals, the central nucleus of the amygdala was severely damaged, with only a very small portion of the dorsal part of the central nucleus remaining intact. In three animals, some parts of the lateral amygdala, the basal amygdala (pars lateralis), and the piriform cortex were slightly damaged. No significant differences in preferential test results amoung these three animals and the other amygdaloid-lesioned animals were observed. Thus, any changes in the preferential behavior after lesioning can be attributed to the damaged central nucleus of the amygdala. According to the atlas of Slotnick and Leonard (17), the largest and the smallest lesion extended from anterior −0.8 to −2.2 mm and anterior −1.2 to −1.9 mm, respectively.

Behavioral results revealed that normal and sham-operated mice showed preference for the morphine-associated stimulus whereas amygdaloid-lesioned mice failed to discriminate between the stimulus mice. Paired *t*-tests (two-tailed) were conducted between the baseline and the CIP test day results for all subject mice. It was shown that the normal mice significantly preferred the morphine-associated stimulus mouse over the

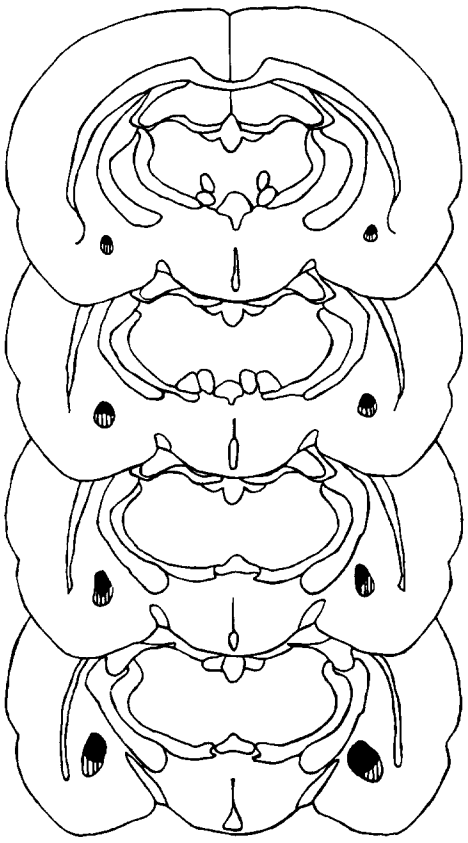

FIG. 1. Drawings of a representative radio frequency lesion. Shaded areas indicate intensive damage and striped areas mean moderate damage at the largest extent. The drawings (top to bottom) correspond to −1.2 mm, −1.5 mm, −1.8 mm. and −2.0 mm plates of the atlas by Slotnick and Leonard (17).

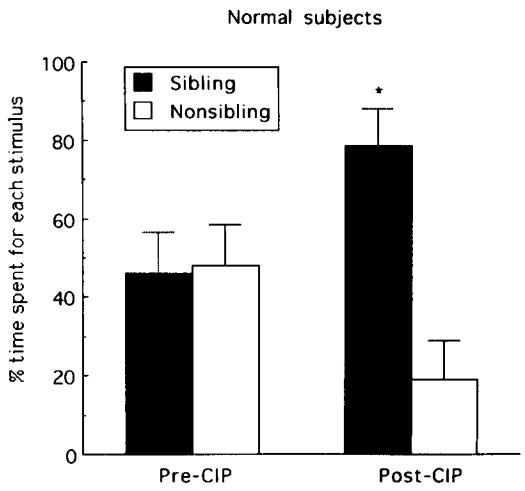


FIG. 2. Mean ± SEM percentage of time spent by normal subjects ($n$ = 10) in the morphine-associated (shaded bar) and the saline-associated (white bar) stimulus during the 15-min test sessions on pre-CIP and post-CIP days. Asterisk indicates significance at $p < 0.01$ (two-tailed $t$-test), $t(9) = 18.12$.

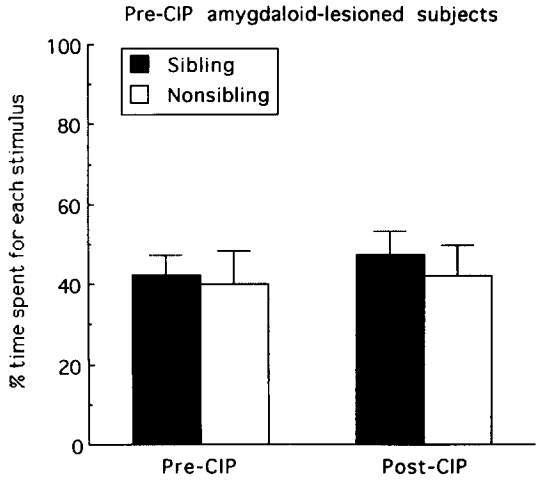


FIG. 4. Mean ± SEM percentage of time spent by subjects receiving bilateral amygdala lesions before CIP training ($n$ = 10) in the morphine-associated (shaded bar) and the saline-associated (white bar) stimulus during the 15-min test sessions on pre-CIP and post-CIP days.

saline-associated stimulus (Fig. 2), $t(9) = 18.12$, $p < 0.01$. Similarly, the sham-operated mice significantly preferred the morphine-associated stimulus (Fig. 3), $t(9) = 26.85$, $p < 0.01$. On the other hand, $t$-test results for mice receiving amygdala lesions before or after the CIP training were clearly nonsignificant (Figs. 4 and 5). A one-factor ANOVA of simple main effects was performed on time spent on the morphine-associated stimulus between groups and revealed significant treatment group differences, $F(3, 36) = 19.1$, $p < 0.01$. An additional independent $t$-test was used to compare the time spent on the morphine-associated stimulus by the amygdaloid-lesioned groups (the data from these two groups were combined) with that of the normal group. A significant statistical difference was observed, $t(18) = 15.2$, $p < 0.01$. These results demonstrate that a significant shift in the time spent in the morphine-associated stimulus had occurred after CIP training in normal mice but not in amygdaloid-lesioned mice.

## DISCUSSION

It was shown here that the amygdala, specifically the central nucleus, mediated the conditioning of individual discrimination in mice. The role of the amygdala was observed to involve the acquisition as well as the expression of this conditioned discrimination. The present results extended a recent finding that the lateral amygdala is primarily involved in the

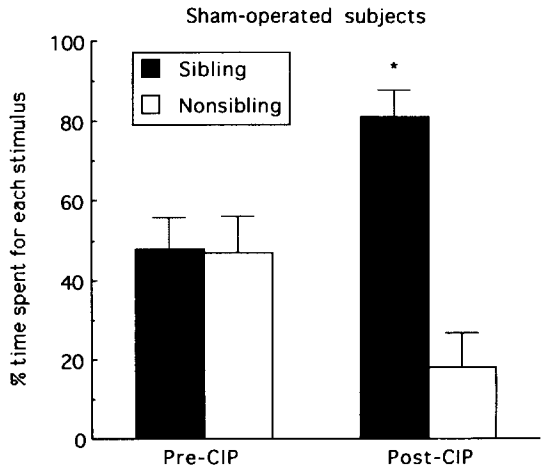


FIG. 3. Mean ± SEM percentage of time spent by sham-operated subjects ($n$ = 10) in the morphine-associated (shaded bar) and the saline-associated (white bar) stimulus during the 15-min test sessions on pre-CIP and post-CIP days. Asterisk indicates significance at $p < 0.01$ (two-tailed $t$-test), $t(9) = 26.85$.

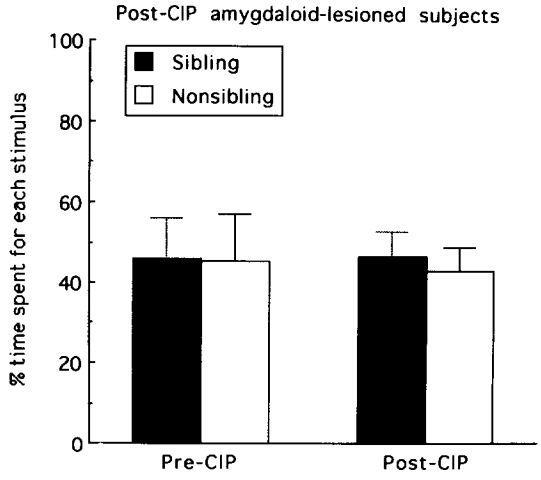


FIG. 5. Mean ± SEM percentage of time spent by subjects receiving bilateral amygdala lesions after CIP training ($n$ = 10) in the morphine-associated (shaded bar) and the saline-associated (white bar) stimulus during the 15-min test sessions on pre-CIP and post-CIP days.

acquisition of the CPP (13). Jellestad and Bakke (8) reported that both lateral and central nuclei of the amygdala appear to be of the output type or relay neurons. This might be the reason for the similarity of the results between the study by McDonald and White (13) and our study.

Our results also confirmed past reports on the possible mediation of opioids in discrimination (1,2,14). The use of morphine here gave further evidence that opioid receptors might be located in the amygdala because the prerequisite for the occurrence of the morphine-conditioned discrimination was an intact amygdala. However, it must be assumed here that the peripheral injection of morphine reached the central nervous system and activated the opiate receptors in the amygdala. Peripherally acting substances have been shown to influence learning and memory centrally by reflex influences on brain activity, through regional changes in cerebral blood flow or through actual penetration into the brain at peculiarly permeable sites (7). Past studies using IP injections of morphine have been shown to induce CPP in rats (10,15). Martin et al. (12) postulated that morphine-like opiates are mediated by $\mu$ receptors. Binding studies show that in guinea pig, the intestine responds more to these $\mu$ receptors [reviewws in (18)]. Thus, the choice of the IP route of morphine administration in the present study was in accord with the above findings. The occurrence of preference can be clearly attributed to morphine's activation of the opiate receptors in the amygdala.

In summary, our study supports the view that damage to the amygdala impairs acquisition of conditioned discrimination (CPP), and also extends this to include conspecific discrimination (CIP). In addition, CIP expression was also shown to be affected by such amygdaloid lesions. The most plausible mechanism for the influence of amygdala to this morphine-induced CIP was via the opioid system. Further research is needed to relate this amygdala opioid system with the other opioid systems that exist in the brain.

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## REFERENCES

1. Bilsky, E. J.; Marglin, S. H.; Reid, L. D. Using antagonists to assess neurochemical coding of a drug's ability to establish a conditioned place preference. Pharmacol. Biochem Behav. 37:425–431; 1990.
2. Blander, A.; Hunt, T.; Blair, R.; Amit, Z. Conditioned place preference: An evaluation of morphine's positive reinforcing properties. Psychopharmacology (Berlin) 84:124–127; 1984.
3. Borlongan, C. V.; Watanabe, S. Conditioned individual preference: Sibling discrimination in mice. (submitted).
4. Borlongan, C. V.; Watanabe, S. Conditioned individual preference: Mother discrimination in mice. (submitted).
5. Borlongan, C. V.; Watanabe, S. Conditioned individual preference: Colony discrimination in mice. (submitted).
6. Carr, G. D.; Fibiger, H. C.; Phillips, A. G. Conditioned place preference as a measure of drug reward. In: Liebman, J. M.; Cooper, S. J., eds. The neuropharmacological basis of reward. Oxford: Clarendon Press; 1989:265–319.
7. Izquierdo, I. Effect of naloxone and morphine on various forms of memory in the rat: Possible role of endogenous opiate mechanisms in memory consolidation. Psychopharmacology (Berlin) 66:199–203; 1979.
8. Jellestad, F. K.; Bakke, H. K. Passive avoidance after ibotenic acid and radio frequency lesions in the rat amygdala. Physiol. Behav. 34:299–305; 1984.
9. Kluver, H.; Barrera, E. A method for combined staining of cells and fibers in the nervous system. J. Neuropathol. Exp. Neurol. 12:400–403; 1953.
10. Kumar, R. Morphine dependence in rats: Secondary reinforcement from environmental stimuli. Psychopharmacologia 25:971–979; 1972.
11. Mackey, W. B.; van der Kooy, D. Neuroleptics block the positive reinforcing effects of amphetamine but not of morphine as measured by place conditioning. Pharmacol. Biochem. Behav. 22: 101–105; 1985.
12. Martin, W. R.; Eades, C. G.; Thompson, J. A.; Huppler, R. E.; Gilbert, P. E. The effects of morphine- and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 197:517–532; 1976.
13. McDonald R. J.; White, N. M. A triple dissociation of memory systems: Hippocampus, amygdala, and dorsal striatum. Behav. Neurosci. 107:3–22; 1993.
14. Mucha, R. F.; Iversen, S. D. Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: A procedural examination. Psychopharmacology (Berlin) 82:241–247; 1984.
15. Rossi, N. A.; Reid, L. Affective states associated with morphine injections. Physiol. Psychol. 4:269–274; 1976.
16. Shippenberg, T. S.; Bals-Kubik, R.; Berz, A. Motivational properties of opioids: Evidence that an activation of $\pi$d-receptors mediates reinforcement processes. Brain Res. 234–239; 1987.
17. Slotnick, B. M.; Leonard, C. M. A stereotaxic atlas of the albino mouse forebrain. Washington, DC: U.S. Government Printing Office; 1975.
18. Snyder, S. H. Drug and neurotransmitter receptors in the brain. Science 22–31; 1984.