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The caudal part of the posterior insula of rats participates in the maintenance but not the acquisition of morphine conditioned place preference

Yong-Mei Sun[1,2] | Rong-Xiang Chen[3] | Zhi-Fei Li[1] | Sabine Spijker[2] | Rong-Wei Zhai[1] | Shang-Chuan Yang[1]

[1]Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences and Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China

[2]Department of Molecular and Cellular Neurobiology, Center for Neurogenomics & Cognitive Research, Amsterdam Neuroscience, VU University Amsterdam, Amsterdam, The Netherlands

[3]Key Laboratory of Magnetic Resonance in Biological Systems, State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan, China

Correspondence
Yong-Mei Sun, Department of Molecular and Cellular Neurobiology, Center for Neurogenomics & Cognitive Research, Amsterdam Neuroscience, VU University Amsterdam, Amsterdam, The Netherlands.
Email: y.m.s.sun@vu.nl

Summary

The heterogeneous insular cortex plays an interoceptive role in drug addiction by signaling the availability of drugs of abuse. Here, we tested whether the caudal part of the multisensory posterior insula (PI) stores somatosensory-associated rewarding memories. Using Sprague Dawley rats as subjects, we first established a morphine-induced conditioned place preference (CPP) paradigm, mainly based on somatic cues. Secondly, an electrolytic lesion of the caudal portion of the PI was carried out before and after the establishment of CPP, respectively. Our data demonstrated that the caudal PI lesions disrupted the maintenance, but not the acquisition of morphine-induced CPP. Lesion or subtle disruption of the PI had no major impact on locomotor activity. These findings indicate that the caudal portion of the PI might be involved in either the storage or the retrieval of morphine CPP memory.

KEYWORDS

conditioned place preference, memory maintenance, morphine, posterior insula, somatosensory

1 | INTRODUCTION

Recently, the insular cortex, or the insula, has gained particular interest for the study of drug addiction because of its possible role in mediating drug use and craving.[1-4] The insula is structurally divided into the anterior viscerosensory/agranular insula (AI) and the posterior somatosensory/granular insula (PI) in humans[1] and rats.[2] Whereas the anterior insula (AI) is widely correlated with drug craving,[3,4] the role of the posterior insula (PI) in drug addiction is still unclear.

The PI, together with the primary somatosensory cortex (S1) and the secondary somatosensory cortex (S2), processes somatic information relayed by thalamic nuclei from lower brain stem nuclei. Somatic information comprises the senses of touch, temperature, pain, and body position,[5] and the PI has been proposed to be involved in the last step of this somatosensory processing pathway.[6] Previous studies in rats supported the equivalence of S1 and S2 in processing unisensory information[7] and the multisensory integration function of the PI.[8-10] Sacco and Sacchetti established that the secondary visual, acoustic, and olfactory cortices, which have multisensory integration functions such as the PI, supported memory storage, and retrieval of sensory stimuli that have acquired a behavioral and emotional salience.[11] Their findings suggest that the PI has a similar function in memory storage. Recently, several studies showed the importance of the more anterior part of this anatomical pathway in the addictive properties of morphine.[12-14] One study from our

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laboratory[13] showed that S1 and S2 are necessary for the acquisition, but not the maintenance, of morphine conditioned place preference (CPP) memory. Similarly, the more rostral part of the posterior granular insula is necessary for the acquisition of morphine CPP memory[14] and expression of morphine CPP.[12] The more caudal part of the PI is known for its role in the maintenance of allodynia by otherwise innocuous sensory stimuli.[15] The caudal part could therefore represent a storage site for somatosensory-related memory with high emotional value. However, its role in relation to the maintenance of addictive memories has not been determined.

Previous studies have suggested a role of the PI in the maintenance of drug-induced memories. Amphetamine-induced CPP induced neuronal activation throughout the granular portion of the PI, from rostral to caudal.[16] Moreover, temporary inactivation of the rostral PI cortex by lidocaine transiently blocked the expression of amphetamine-induced CPP. In contrast, the AI, which receives mostly thalamic and amygdalar input, seems to have a limited role in the maintenance of drug-induced CPP, and might relate more to reconsolidation of the drug memory.[17] Moreover, lesion of the AI has no effect on either the acquisition[18] or the expression[19] of morphine-induced CPP. Taken together, the PI might play a crucial role in the expression of morphine-related memories.

Consequently, we hypothesize that the most caudal part of the PI participates in the expression, but not the acquisition of morphine-induced CPP. To test this hypothesis, the caudal part of the PI was electrically lesioned before and after the establishment of CPP.

2 | MATERIALS AND METHODS

2.1 | Subjects

Sixty Sprague Dawley male adult rats weighing 220-240 g upon arrival were obtained from the Zigong Animal Laboratory (Sichuan, China) and housed in groups of four to six in a temperature-controlled (23 ± 2°C) animal facility. The rats were maintained on a 12-h light/dark cycle (lights on from 7:00 AM to 7:00 PM) with free access to food and water and were handled twice per day for a week before the experiments. The experimental procedures were performed in accordance with the guidelines for the National Care and Use of Animals, and the experiments were approved by the Institutional Animal Care and Use Committee of Kunming Institute of Zoology. All efforts were made to minimize animal suffering and to reduce the number of animals required.

2.2 | Drugs

Morphine hydrochloride (Shenyang First Pharmaceutical Factory, Shenyang, China) is a 10 mg/mL per ampule and was given intraperitoneally (ip) as 1 mL/kg to rats. Chloral hydrate (Sinopharm Chemical Reagent Company, Shanghai, China) was dissolved into 0.9% sterilized saline and stored in 10% concentration and was given as 400 mg/kg to rats. Gentamycin sulfate injection (Huai qing tang Company, Henan, China) was 40 000 units/mL. Injections were given intraperitoneally (ip), 0.25 mL per rat.

2.3 | Surgery and electrolytic lesions

Rats, weighing 280-320 g upon the surgery, were first treated with atropine (0.4 mg/kg body weight) to reduce mucous secretion, anesthetized with an intraperitoneal injection of 10% chloral hydrate (400 mg/kg body weight), and mounted in a stereotaxic apparatus (RWD Life Science Company, San Diego, United States). Body temperature was maintained at normothermia using a heating pad. The scalp was incised and retracted, and the head position was adjusted to place the bregma and lambda in the same horizontal plane. Small burr holes (1 mm in diameter) were drilled bilaterally in the skull (anterior-posterior −1.7 mm, medial-lateral ±5.0 mm), and previously prepared silver electrodes (0.35 mm in inner diameter, 0.5 mm of the tips was not covered by insulating sleeve) were implanted 6.0 mm with a vertical angle of 15° to target the caudal part of the PI. A direct current of 1 mA (brain as anode and exposed skin as cathode) was given for 30 s on each side, as this was the most optimal time for creating a lesion without more than 10% collateral damage. Animals in the sham groups were treated with the same manipulation as true lesion groups but without current exposure. Rats, with lesions of the caudal part of the PI and less than 10% percent of adjacent somatosensory cortices, were included in this study. In total, seven rats were discarded for not meeting these criteria. After the surgery, gentamycin (8000 units/rat) was injected (*ip*) to minimize infection.

2.4 | Conditioned place preference apparatus and paradigm

2.4.1 | Apparatus

The apparatus for training and testing consisted of four identical three-chamber polyvinyl chloride (PVC) boxes, separated by two removable guillotine doors. Two large black side chambers (30 cm long × 25 cm wide × 30 cm high) differed in floor texture (one with a grid plexiglass floor, the other with a rough PVC floor), and were connected by a smaller white box (11 cm long × 25 cm wide × 30 cm high with a smooth white PVC floor). Previous experimental data showed that although a group of rats (n = 20) had no preference for the two side chambers,[20] individual animals showed bias. Therefore, for this study, we used a balanced biased design with saline controls.[21] The nonpreferred chamber in the saline group was used to calculate the CPP score in the saline group. The activity of rats was recorded using a video camera mounted to the ceiling, 1.5 m above the center of the CPP apparatus. The information regarding the activity of the rats was transferred to a computer in a separate room for offline analysis. Rats were considered to have entered a chamber when the head and two front paws were inside the chamber. The locomotor activity during testing was measured in a low-level manner by counting entrances to the morphine-paired chamber and saline-paired chamber, respectively.

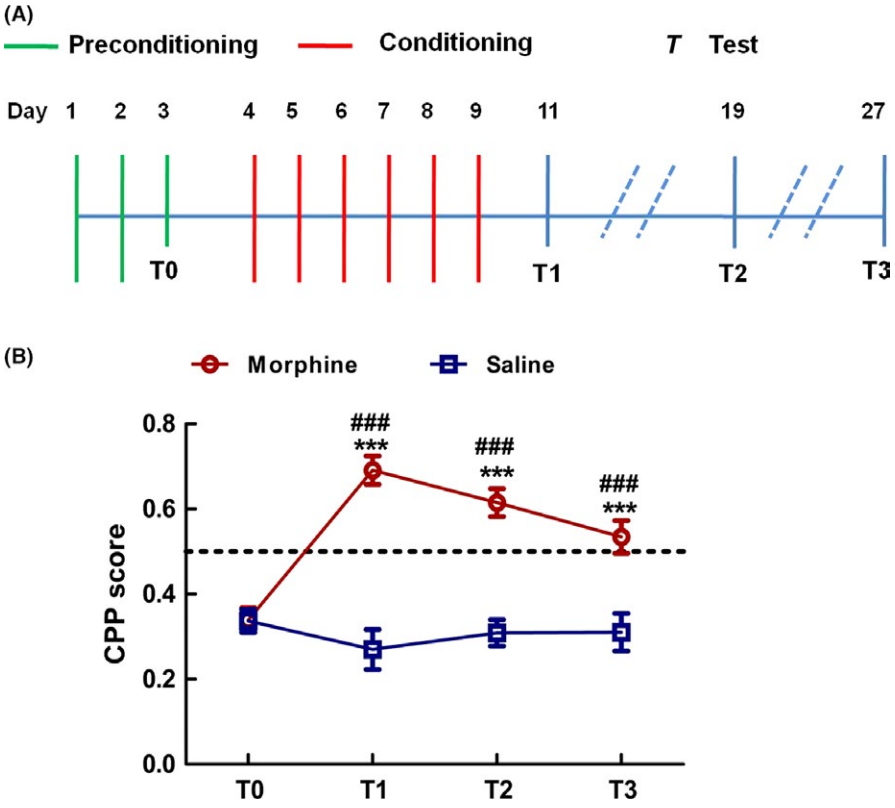

FIGURE 1 Morphine conditioned place preference (CPP) memory can last at least 18 d. A, The overview of the experimental schedule in the morphine (n = 13) and saline (n = 10) groups. B, Memory retention of CPP in the morphine and saline groups was assessed by measuring the CPP score at several time points (blue line in schedule) consecutively after the last conditioning day (red lines in schedule), which were 2 d (T1), 10 d (T2), and 18 d (T3) after the last conditioning session. All values of CPP score are presented as mean ± SEM. The CPP score represents the index of place preference for each rat, calculated by dividing the time spent in the drug-paired chamber/nonpreferred chamber by the total time spent in both conditioning chambers. ###$P < 0.001$ compared with the saline group; ***$P < 0.001$ compared with T0 in the morphine group

2.4.2 | Behavioral training and testing of the CPP paradigm

The morphine-induced CPP paradigm was established according to previous studies .[22,23] A short description is given below.

2.4.3 | Preconditioning phase

During this phase (days 1-3), each animal, irrespective of being in the morphine or saline group, was placed in the middle chamber with guillotine doors removed to allow free access to the entire apparatus for 15 minutes. The time spent in the two side chambers on day 3 was calculated as the baseline and referred to as T0. Rats that entered less than four times to either of the side chambers in the preconditioning phase were removed from the experiment[13]; four rats were excluded based on this criterion.

2.4.4 | Conditioning phase

The conditioning phase consisted of 6 days (days 4-9). For morphine-conditioned subjects, intraperitoneal injections of morphine (10 mg/kg) or saline were given alternately in the morning (9:00 AM) and evening (7:00 PM) sessions (10 hours apart), and each injection was followed by immediate confinement of the rat to its drug-paired or saline-paired chamber for 45 minutes. After receiving morphine, rats were confined to the nonpreferred chamber. For saline-conditioned controls, rats were given only saline prior to exposure to one chamber of the CPP apparatus during the morning session and to the other side during the evening session for 45 minutes per session. Injections were counterbalanced for the injection time of the day.

2.4.5 | Testing phase

Three postconditioning tests, that is, test 1 (T1), test 2 (T2), and test 3 (T3), were carried out in a morphine-free state on day 2, day 10, and day 18 after the last conditioning session, respectively.

The CPP score represents the index of place preference for each rat with morphine conditioning experiences, calculated by dividing the time spent in the drug-paired chamber by the total time spent in both conditioning chambers. In the saline group, the CPP score was calculated by the time spent in the nonpreferred chamber by the total time spent in both conditioning chambers.

2.5 | Experimental design

2.5.1 | Establishment of morphine-induced CPP

Rats in the morphine group (n = 13) were used to test the establishment of morphine CPP (Figure 1A). As we used the biased design of the CPP paradigm, ten rats in the saline group were only given saline during the conditioning process.

2.5.2 | Effect of pre-CPP PI lesion

To test whether the caudal part of the PI participates in the acquisition of CPP, lesions in the LCPP group (n = 6) and sham lesions in the

ShamLCPP group (n = 6) were carried out 7 days before the first preconditioning day (day 1) (Figure 3A).

2.5.3 | Effect of post-CPP PI lesion

To test whether PI is necessary for the expression of CPP, rats in the CPPL (n = 8) and CPPShamL (n = 6) groups were lesioned and sham-lesioned 1 day after T1 (day 12) (Figure 4A).

2.6 | Histology

After completion of behavioral testing, rats were deeply anesthetized with 10% chloral hydrate (450 mg/kg, i.p.) and then perfused through the left ventricle of the heart with a saline flush (100 mL) followed by 300 mL of 4% paraformaldehyde in phosphate-buffered saline (PBS) (pH = 7.4). The brains were postfixed in the same fixative for 2 hours and first transferred to 20% sucrose and then to 30% sucrose in PBS until they sank. Lesion areas were assessed using Nissl staining[14] (20 μm per slice) and a light microscope (Olympus CX41), and as shown in Figure 2D, only the animals with minimal and maximal area of lesion were further analyzed in more detailed histological reconstruction.

2.7 | Statistical analysis

All behavioral data were presented as mean ± SEM. CPP scores and entrances to drug-paired compartments were analyzed using paired *t* tests and analysis of variance (ANOVA) with the appropriate between- and within-subject factors. The statistical assumptions (outliers, homogeneity of covariance and variance, normality, and sphericity) were controlled for the different experiments (see Results section). Post hoc analyses of significant effects in the ANOVA were performed using the Bonferroni test or least significant differences test (LSD); significant differences are indicated by asterisks (*) and octothorpesin (#) in the figures. Values of $P < 0.05$ were considered statistically significant.

3 | RESULTS

We used the CPP paradigm to study reward-related learning and memory of a morphine-paired context.[24,25] In our experimental setup, we either lesioned the caudal portion of the PI 7 days before preconditioning with morphine and tested animals on day 2 (T1) after the last conditioning (Figure 3A), or lesioned the caudal part of the PI 1 day after T1, and tested these animals 1 week after the lesion (T2) (Figure 4A).

3.1 | Histology

The electrolytic PI lesions were comparable between the two groups, that is, the lesion before and after the establishment of CPP. Based on the brain atlas, we show a schematic representation of the targeted

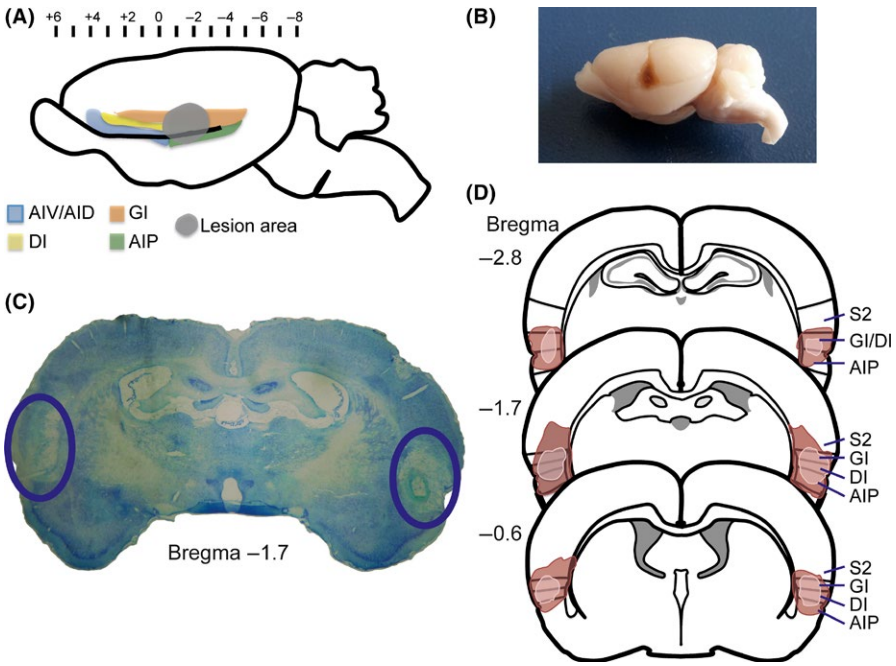

FIGURE 2 Lesion verification. A, Schematic drawing of the insular cortex highlighted with subregions indicated in color. The black line going through the insular area is the rhinal fissure. The gray area depicts the targeted lesion area. AIV/AID, agranular insular cortex ventral/dorsal; AIP, agranular insular cortex, post; DI, dysgranular insular cortex; GI, granular insular cortex. B, An example of the lesion area of the left insula. The dark area is the targeted lesion area. The black line, extending from the olfactory bulb to the caudal part of the cerebral cortex, is the rhinal fissure. C, Nissl stain verification of the lesioned areas, as indicated by blue circles. D, Histological reconstruction of the smallest (light pink) and largest (dark pink) electrolytic lesions aiming at the caudal portion of the PI, consisting of the DI, AIP, and GI; S2, secondary somatosensory cortex. Negative numbers indicate posterior distance from the bregma. Pictures were adapted from the atlas of Paxinos and Watson (2007)

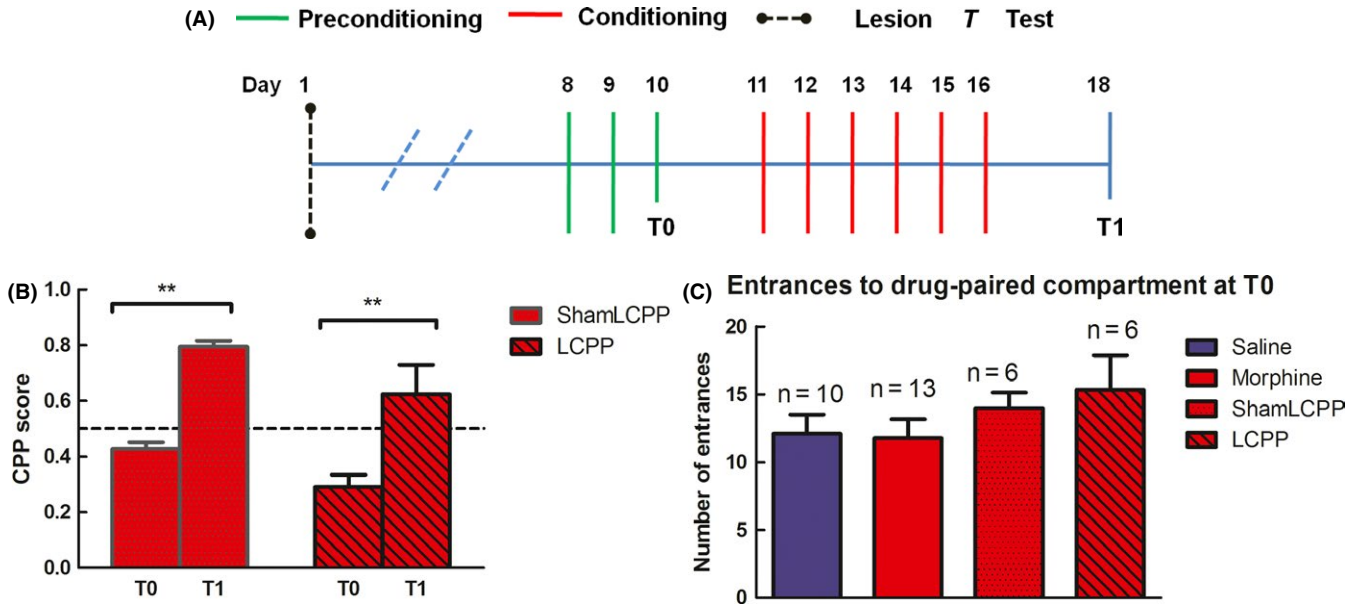

FIGURE 3 Lesion before conditioned place preference (CPP) had no effect on the establishment of CPP. A, Scheme of experimental design, with lesion (day 1), pretest (T0, day 10), and post-test (T1, day 18) indicated. B, Establishment of CPP in the LCPP group (two-tailed *t* test, $t(5) = 3.857$, $P = 0.01$) and ShamLCPP group (two-tailed *t* test, $t(5) = 23.321$, $P < 0.01$). C, Despite the initial difference in pre-CPP score (T0) of the ShamLCPP and LCPP groups, the locomotor activity measured as the number of entrances at this time point of all groups was not different (one-way ANOVA, $F(3,31) = 0.953$, $P > 0.05$). $^{**}P < 0.01$

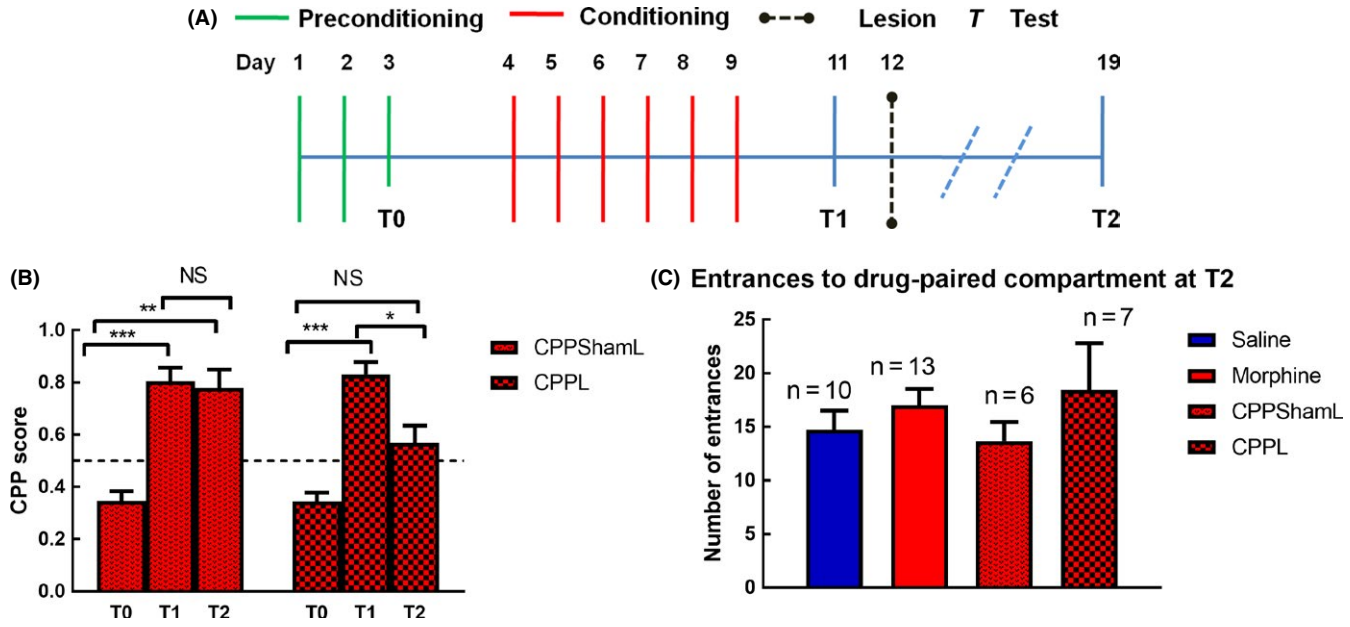

FIGURE 4 Lesion of the caudal part of the posterior insula after conditioned place preference (CPP) impaired the expression of CPP but not locomotor activity. A, Scheme of experimental design, pretest (T0, day 3), post-test (T1, day 11), and lesion (day 12) indicated. B, Lesion after the establishment of CPP impaired the maintenance of CPP measured 7 d later. C, Lesion had no effect on the locomotor activity measured as the number of entrances. All values are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NS, no significance

lesion area with the whole insular area as background (Figure 2A), as well as the actual lesioned area (Figure 2B). Although in all cases the PI was hit, in some rats there was extensive damage to the adjacent somatosensory cortices (Figure 2C,D). As S1 and S2 were shown to play a role in the acquisition of morphine-induced CPP,[13] the rats that had <10% of somatosensory lesions were used in the experiments.

3.2 | Behavior

3.2.1 | Establishment of morphine-induced CPP

Firstly, 23 rats were used to establish morphine-induced CPP and three tests, that is, 2 days (T1), 10 days (T2), and 18 days (T3)

after last conditioning session were carried out to test the maintenance of morphine CPP (Figure 1A). A mixed ANOVA with group (saline vs morphine) as a between-subjects factor and test (T0, T1, T2, and T3) as a within-subjects factor revealed that there were significant differences in the interaction between group and test [$F(3,63) = 16.335$, $P < 0.001$], test [$F(3,63) = 8.383$, $P < 0.001$], and group [$F(1,21) = 50.061$, $P < 0.001$]. Thus, a subsequent one-way repeated-measures ANOVA was performed and the results revealed a significant difference among tests [$F(3,36) = 25.239$, $P < 0.001$] in the morphine-treated group, which was absent in the saline-treated group [$F(3,27) = 0.793$, $P = 0.508$]. The multiple comparison analysis with Bonferroni adjustment in the morphine group showed significant differences between T1 and T0 ($P < 0.001$), T2 and T0 ($P < 0.001$), and T3 and T0 ($P = 0.009$), as in all three tests the rats showed preference for the morphine-paired compartment. These results showed that the maintenance of morphine CPP lasted at least 18 days after the last conditioning session (Figure 1B).

3.2.2 | Lesion before CPP had no effect on the establishment of CPP

To test the role of the caudal part of the PI in the acquisition of CPP, we lesioned and sham-lesioned the PI 7 days before the preconditioning phase (Figure 3A), and compared the lesion (LCPP) group with a sham-lesioned group (ShamLCPP). A mixed ANOVA with group (LCPP vs ShamLCPP) as a between-subjects factor and test (T0 and T1) as a within-subjects factor revealed a test effect [$F(1,10) = 63.862$, $P < 0.001$], but no interaction effect [$F(1,10) = 0.176$, $P = 0.684$] (Figure 3B). The trend for a group effect [$F(1,10) = 4.348$, $P = 0.064$] was due to higher overall CPP scores (pre- and postconditioning) in the sham lesion group compared with the lesion group (Table 1). Despite this difference, locomotor activity between the LCPP and ShamLCPP group was similar to that of the morphine and saline groups at the pretest T0 (Figure 3C, one-way ANOVA, $F(3,31) = 0.953$, $P = 0.427$), indicating that the PI lesions had no effect on the animals' locomotor activity.

3.2.3 | Lesion after CPP impaired the expression of CPP

To test whether the caudal part of the PI is required for the maintenance of morphine CPP, we lesioned the PI after the establishment of CPP (Figure 4A), that is, 1 day after T1, and compared the lesioned (CPPL) and sham-lesioned (CPPShamL) groups. A mixed ANOVA with group (CPPL vs CPPShamL) as a between-subjects factor and test (T0, T1, and T2) as a within-subjects factor revealed a significant test effect [$F(2,22) = 53.253$, $P < 0.001$] and a test-by-group interaction [$F(2,22) = 3.770$, $P = 0.039$], but no group effect [$F(1,11) = 1.492$, $P = 0.247$] (Figure 4B). Subsequently, a one-way repeated-measures ANOVA for each group revealed significant differences of CPP scores for time in both the CPPL [$F(2,12) = 23.343$, $P < 0.001$] and CPPShamL [$F(2,10) = 39.799$, $P < 0.001$] groups. Further Bonferroni comparisons showed that in the control CPPShamL group and the CPPL group alike, the CPP scores at T1 were significantly higher than T0, showing no between-group differences before the lesion took place. In the control CPPShamL group, the CPP score remained elevated at T2, as it was shown that the "morphine reward" memory lasted 10 days after the last conditioning session (Figure 1B). However, in the CPPL group (Figure 4B), there was a significant decrease in the CPP score at T2 compared with T1 ($P = 0.022$), indicative of the lesion effect on the maintenance of morphine CPP. CPP scores at T2 were marginally higher than that at T0 ($P = 0.088$), further validating that the lesion significantly attenuated memory for the morphine-associated context. Table 2 shows that individual differences existed in both the acquisition of CPP and the effect of the lesion (including sham lesion) on the expression of the morphine CPP in the CPPL and CPPShamL groups.

However, the decrease in the CPP score was not accompanied by changes in locomotor activity (Figure 4C). The CPPL and CPPShamL groups showed equal entries into the drug-paired compartment when compared with the morphine and saline groups [$F(3,32) = 0.708$, $P = 0.554$] during the CPP T2. Moreover, the CPPShamL group showed no difference to the morphine group ($P = 0.341$). Together, these results showed that a lesion of the caudal portion of the PI disrupted the maintenance, but not the acquisition of morphine CPP, and had no effect on locomotor activity.

TABLE 1 Individual conditioned place preference (CPP) scores for LCPP and ShamLCPP groups that received lesions prior to conditioning. This table shows the differences at the individual level

Rat number	Group	T0 CPP score	T1 CPP score
1	LCPP	0.46	0.83
2	LCPP	0.29	0.74
3	LCPP	0.21	0.65
4	LCPP	0.31	0.36
5	LCPP	0.15	0.25
6	LCPP	0.32	0.90
7	ShamLCPP	0.37	0.80
8	ShamLCPP	0.47	0.80
9	ShamLCPP	0.43	0.83
10	ShamLCPP	0.50	0.84
11	ShamLCPP	0.41	0.77
12	ShamLCPP	0.36	0.71

4 | DISCUSSION

This study focused on the somatosensory-associated learning and memory function of the PI in addiction, a brain area that has been implicated in drug addiction with some controversy.[26] To explore whether the caudal part of the PI participated in the acquisition or expression of somatosensory-associated reward memory, we electrically lesioned the PI before and after the establishment of morphine-induced CPP.

TABLE 2 Individual conditioned place preference (CPP) scores of the post-CPP lesion and sham lesion groups. This table shows the differences at the individual level

Rat number	Group	T0 CPP score	T1 CPP score	T2 CPP score
1	CPPL	0.29	0.91	0.58
2	CPPL	0.40	0.92	0.81
3	CPPL	0.31	0.93	0.31
4	CPPL	0.32	0.51	0.27
5	CPPL	0.44	0.76	0.56
6	CPPL	0.47	0.91	0.75
7	CPPL	0.18	0.86	0.70
8	CPPShamL	0.31	0.83	0.79
9	CPPShamL	0.25	0.84	0.84
10	CPPShamL	0.29	0.74	0.86
11	CPPShamL	0.37	0.86	0.84
12	CPPShamL	0.39	0.89	0.78
13	CPPShamL	0.48	0.67	0.56

4.1 | Effect of pre-CPP PI lesion

Our results showed that lesion of the PI had no effect on the acquisition of morphine CPP memory. There are at least two possible explanations for this. The first is that the caudal part of the PI does not have a function in reward memory acquisition. Even though our lesion caused damage to the adjacent somatosensory cortices, the criteria used, with <10% lesion of adjacent sites, apparently limited their impact on the acquisition of morphine-induced CPP, as we did not observe a difference between treatment groups. In the visual and auditory cortices, the primary cortices are responsible for the acquisition of associative memories, while the secondary cortices are crucial for the storage of associative memories.[11] Likewise, Meng et al[13] have shown that the somatosensory cortices participate in the formation but not storage of morphine-induced associative memory. Our data indicate that the caudal PI did not participate in the acquisition of morphine-induced associative memory. This implies that the PI has a higher somatosensory function, which is consistent with previous studies.[27,28]

Another possibility is that the effect of the lesion of the caudal PI is masked by the lesion of the surrounding somatosensory cortices and the rostral part of the PI. As these brain regions are necessary in the acquisition of morphine, their lesioning would lead to a diminished CPP score.[13,14] Human image studies showed that drug-dependent heroin and cocaine patients had reduced gray matter in the right PI,[6,29] and this was suggested as a possible marker of increased vulnerability to drug addiction. Reasoning along this line, the lesion of the PI we induced might increase the vulnerability to drug abuse. If so, the lesion of the caudal part of the PI would increase morphine CPP. As yet, we are unable to make this distinction regarding the role of the caudal part of the PI with respect to reward memory acquisition.

With respect to the timing of the lesion operation, we based ourselves on a previous study from our laboratory.[13] Albeit that the CPP scores of the post- and pretest were lower in the lesioned group compared with the sham-lesioned group (Table 1), the relative increase was similar. As yet, we cannot discern whether there are any confounding effects, but future studies would likely benefit from taking a longer postsurgery recovery time (eg, 2 weeks).

Other studies in rats showed that the PI was necessary for the acquisition of CPP,[12,14,17] which is seemingly in conflict with our result. However, these studies investigated either the rostral part[14,17] or the medial part[12] of the PI, whereas we focused on the caudal part of the PI. Together, these results suggest that different parts of the PI have different functions in drug addiction.

4.2 | Effect of post-CPP PI lesion

Previous studies showed that the insular cortex is necessary in drug craving and seeking behaviors in rats.[16,30] However, one study[18] showed that lesioning of the visceral insula, that is, anterior insula, did not disrupt the acquisition of morphine place preference. Therefore, it is very important to clarify which part is responsible for which phase in the process of drug addiction. In our study, we found that lesioning of the caudal part of the PI after the establishment of CPP impaired the maintenance of morphine-induced CPP. Furthermore, this lesion did not affect locomotor activity measured in a low-level gage of rats. These findings suggest that the PI may play a role in the storage of somatosensory-associated drug-rewarding memory. Our findings are consistent with other studies. In humans, damage to the insula disrupts addiction to cigarette smoking.[31] Similarly, inactivation of the PI in rats might block the expression of amphetamine-induced CPP.[16] However, it might also be possible that the PI is an upstream site of the drug craving pathway. Many functional imaging studies have revealed activation of the insula during drug urges.[32] Based on human neuroimaging studies, the AI was proposed to be the ultimate site in the drug craving pathway.[4] In rodent studies, the AI plays a role in drug craving[16] and drug memory reconsolidation,[17] while the medial PI participated in drug memory reconsolidation[17] and expression of CPP.[12] Furthermore, as the AI receives projections from the PI,[28] it might be that upon drug craving and expression of the drug memory, neurons are first activated in the caudal part of the PI, then the medial part, and finally the anterior part.

Another explanation is that the caudal part of the PI in rats might be part of a separate pathway involved in storage of drug-related memories due to its connection with the basolateral amygdala. As previous studies showed, this brain area directly projects somatosensory-related inputs to the basolateral amygdala,[28,33] which has been shown to be involved in drug-related memories.[34-36]

4.3 | Concluding remarks

Taken together, our results suggest that the caudal part of the PI is necessary in the maintenance of morphine CPP memory. As this brain region has a higher somatosensory function,[37] our data might imply

that somatosensory might be a critical element of the morphine CPP memory.

We used electrolytic lesions to investigate the brain function in morphine reward. It is known that electrolytic lesions often damage the neuronal structure and the axons passing through the area. Although we demonstrated that the caudal PI was necessary for the maintenance of CPP, as yet we cannot specify whether the PI or fibers passing through this area are involved in this process. However, future studies can apply immunohistochemical methods (eg, c-fos, delta Fos B), together with pharmacological and optogenetic manipulations to verify which kind of neurons in this brain area participates in the maintenance of morphine CPP memory. Albeit that the size of the lesion was large, a previous study from our laboratory[13] showed that a similar, or even larger lesioned area when placed in S1 and S2, had an effect on CPP acquisition. These somatosensory cortical lesions therefore served as an anatomical specificity reference point in our current design.

On the basis of our current data, it will be of interest to study the effect of a PI lesion on morphine-induced and cue-induced reinstatement in future studies. Furthermore, as the PI is indeed not required for the acquisition but the maintenance of morphine CPP, it would be interesting to further investigate which brain region compensates for the PI's acquisition function. In addition, it would be meaningful to perform a more fine-grained analysis of locomotor activity to reflect the anxiety of animals during the expression of CPP and during conditioning, by analyzing distance traveled and area covered, apart from the rough analysis of entrances to the conditioning chamber as performed now.

Using morphine-induced CPP with somatosensory cues, our results showed that the caudal part of the PI participated in the expression, but not the acquisition of morphine CPP. These findings further support a somatosensory-associated memory maintenance role of the caudal part of the PI.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

ORCID


Yong-Mei Sun http://orcid.org/0000-0003-0488-5136

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