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Effects of inactivating the agranular or granular insular cortex on the acquisition of the morphine-induced conditioned place preference and naloxone-precipitated conditioned place aversion in rats

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Abstract

Recent studies have indicated that the insula underlies affective learning. Although affective learning is well-established in the development of opiate addiction, the role of insula in this context remains unclear. To elucidate the organization of opiate-related affective learning within the insular cortex, we reversibly inactivated each of two major subdivisions of the insula in rats and tested the effects of this inactivation on the acquisition of morphine-induced conditioned place preference (CPP) and conditioned place aversion (CPA) induced by naloxone-precipitated acute morphine withdrawal. Results showed that inactivation of the primary interoceptive posterior granular insula (GI), but not that of the high-order anterior agranular insula (AI), disrupted the acquisition of CPP and that both GI and AI inactivation impaired the acquisition of CPA. These data suggest that the insular cortex is involved in positive and negative affective learning related to opiate addiction. In particular, the GI appears to be critical for both forms of affective learning, whereas the AI is crucial for learning associated with negative affects induced by opiate withdrawal.

Keywords

Morphine, naloxone, agranular, granular, insular cortex, conditioned place preference, conditioned place aversion

Introduction

Affective learning plays an important role in opiate addiction. For example, opiates induce positive affective states, whereas cessation of drug use in opiate abusers produces negative affective states such as irritability, anxiety, and dysphoria (O'Brien, 2008; Schulteis and Koob, 1996). Both positive and negative affective effects reinforce drug-seeking behavior through affective learning, at least at the initial stages of addiction (Baker et al., 2004; Schulteis and Koob, 1996).

Evidence indicates that the insula, in addition to the well-established amygdala and nucleus accumbens, regulates affective learning (Ferreira et al., 2005; Koh and Bernstein, 2005; Miranda et al., 2008; Schulteis and Koob, 1996). For example, disruption of long-term potentiation (LTP) in the insula impairs the acquisition of conditioned taste aversion (CTA), a well-established paradigm of affective learning (Escobar et al., 1998). Recently, in vivo optical imaging provided more direct evidence. That is, after training in CTA, insular representation of a pleasant stimulus became similar to an unpleasant one (Accolla and Carleton, 2008). These results support the view that the insula underlies affective learning. However, it is not clear how the insula contributes to opiate-related affective learning during the development of opiate addiction.

Moreover, previous studies have implied that subdivisions of the insula may differentially contribute to opiate-related affective learning. Lesion studies suggest that the agranular insula (AI) area is involved in negative, but not postive, opiate-related affective learning. For example, lesions of the AI impaired the acquisition of morphine-induced CTA (Lin et al., 2009; Mackey et al., 1986; Zito et al., 1988) but had no effect on the acquisition of morphine-induced conditioned place preference (CPP) (Mackey et al., 1986). On the other hand, a recent study suggested that the granular insula (GI) may be involved in drug-related positive affective learning because hypocretin-1 receptors in the GI mediate the rewarding properties of nicotine (Hollander et al., 2008). Furthermore, neural connections of the insula also suggest functional heterogeneity in opiate-related affective learning. For example, the posterior GI receives visceral inputs from the thalamus and parabrachial nuclei (Allen et al., 1991; Cechetto, 1987), suggesting a role as a primary interoceptive area. On the other hand, the anterior AI may be a high-order interoceptive cortex, because it connects with the GI and the limbic structures such as the amygdala and nucleus accumbens (Ohara et al., 2003). Based

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on these data, we hypothesized that the GI and AI of the insula may differentially contribute to affective learning related to opiate addiction.

To test this hypothesis, the present study compared the effects of local chemical inactivation of the AI and GI on opiate-related affective learning in rats. We chose morphine-induced CPP and conditioned place aversion (CPA) induced by naloxone-precipitated acute morphine withdrawal as behavioral paradigms of positive and negative affective learning, respectively (Azar et al., 2003; Bardo and Bevins, 2000). A separate group of rats were examined in the Morris water maze to assess possible alteration of general motor and/or spatial learning abilities after inactivation of the AI/GI.

Materials and methods

Subjects

Male Sprague-Dawley rats (250–280 g) (Vital River Laboratory Animal Technology Co. Ltd, Beijing, China) were used. All rats were individually housed in a colony room with controlled temperature (20–24°C) and humidity (40–70%) on a 12 h/12 h light/dark cycle. Food and water were available ad libitum. All experiments were conducted in the light phase (08:00–18:00). All experimental protocols and procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and the Regulations for the Administration of Affairs Concerning Experimental Animals (China, 1988). The experimental protocol was approved by the Research Ethics Review Board of Institute of Psychology, Chinese Academy of Sciences.

Surgery

Rats were anesthetized with sodium pentobarbital (60 mg/kg, intraperitoneally (i.p.)) and placed in a stereotaxic apparatus (Stoelting Company, USA). Stainless steel guide cannula (outer diameter (o.d.) 0.6 mm, inner diameter (i.d.) 0.35 mm, length 9 mm) were implanted bilaterally, ending 1.5 mm above the injection site (AI: bregma +1.2 mm, midline 4.5 mm, 5.5 mm ventral to the skull; GI: bregma –0.36 mm, midline 5 mm, 5 mm ventral to the skull) (Paxinos and Watson, 2005). Following a previous study on insula (Contreras et al., 2007), cannula were implanted with a 10° angle towards the midline to ensure proper placement of the injection cannula into the laterally-located insular cortex of the rat. To prevent occlusion, a stylet was inserted into the guide cannula. All rats were allowed to recover for seven days.

Drugs and microinjections

Morphine hydrochloride (Qinghai Pharmaceutical, China) and naloxone hydrochloride (Sigma, Missouri, USA) were respectively dissolved in sterile physiological saline (0.9% NaCl) and were administered i.p. at volumes of 1.0 mL/kg body weight.

A mixture of gamma-aminobutyric acid (GABA) agonists (baclofen (Bac), $GABA_B$ agonist, and muscimol (Mus), $GABA_A$ agonist, Sigma, Missouri, USA) (Bac-Mus mix) was chosen to reversibly inactivate the insula. The literature showed that the rat insula expresses both $GABA_A$ and $GABA_B$ receptors (Jasmin et al., 2003). GABA agonists reportedly produce a rapid and prolonged reduction in neuronal activity without affecting fibers of passage (Martin and Ghez, 1999; Van Duuren et al., 2007).

Bac-Mus mix was dissolved in sterile physiological saline to a concentration of 0.6 nmol/μL for Bac and 0.06 nmol/μL for Mus (Di Ciano and Everitt, 2004; Forget et al., 2010; McFarland and Kalivas, 2001). The mix was injected bilaterally at a volume of 0.5 μL/side to the AI/GI 10–15 min prior to training. Microinjection was delivered through an injection cannula coupled with a 1.0 μL Hamilton microsyringe, driven by a microinfusion pump (Cole Parmer, IITC, Life Sci. Instruments, California, USA) at the rate of 0.25 μL/min over 2 min. The injector cannula remained in the guide cannula for another minute to prevent backflow.

Apparatus

For the morphine-induced CPP and naloxone-precipitated CPA we used rectangular two-compartment plastic chambers (80 cm×40 cm×50 cm). The two compartments were separated with a guillotine door and had distinctive visual and tactual cues. One compartment had black walls with white stripes and a bumpy floor. The other had black walls and a grid floor. The apparatus was placed in a room dimly lit with three incandescent bulbs (15 W). The location and movement of rats were monitored by a video camera suspended from the ceiling and analyzed for time spent in each compartment using tracking Software (Taiji Software Company, Beijing, China).

The Morris water maze used a pool (1.8 m in diameter and 0.8 m high) filled with water (22±1°C). The pool was placed in the center of a dimly lit room with various salient visual cues. A transparent platform (10 cm in diameter) was hidden 1.5 cm below the water surface in a fixed location in the pool throughout the experiment. A tracking system (Taiji Software Company, Beijing, China) with a video camera suspended from the ceiling automatically recorded and analyzed each animal's behavioral performance. Black paint (Shanghai Ink Factory, Shanghai, China) was added to the water to facilitate tracking of the white rats and to obscure the platform.

Behavioral tests and experimental design

CPP. To examine the acquisition of morphine-related positive affective learning, a morphine-induced CPP procedure was used. Positive affective learning is indexed as an increase in the time spent in morphine-paired side (Rezayof et al., 2007).

The procedure was adapted from previous studies with minor modification (Rezayof et al., 2007; Yim et al., 2006; Yonghui et al., 2006). As illustrated in a diagram of the experimental protocol (Figure 1(a)), the procedure consists of four phases: adaptation (day 1), pre-test (day 2–3), conditioning (days 4–9), and post-test (day 10).

On adaptation day, each rat was allowed 15 min to move freely in the apparatus to reduce novelty-induced anxiety. On both pre-test days, each rat was placed in the previously-explored apparatus for 15 min to assess individual initial preference.

On conditioning days (days 4–9), a biased CPP design was used. That is, for each rat, morphine was paired with the compartment that it spent less time in (non-preferred side) during the pre-test period. Rats were injected with morphine (5 mg/kg, i.p.) and

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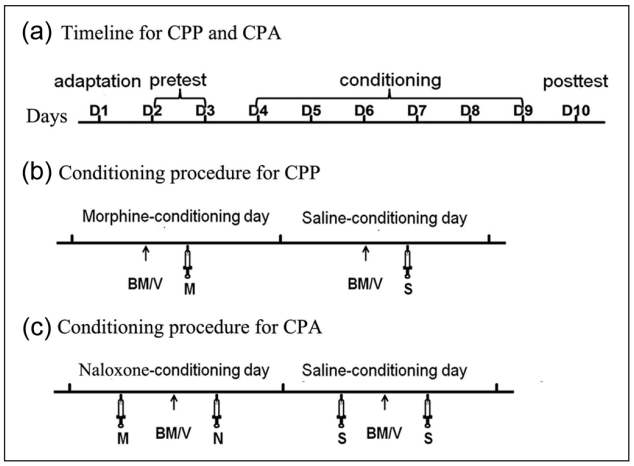

Figure 1. Diagram showing (a) the timeline and the sequence of treatments of (b) the morphine-induced conditioned place preference (CPP) and(c) naloxone-precipitated conditioned place aversion (CPA). BM/V: baclofen-muscimol mixture/ vehicle; M: morphine; N: naloxone; S: saline.
D1–D10 represents experimental days 1 to 10 in each procedure.

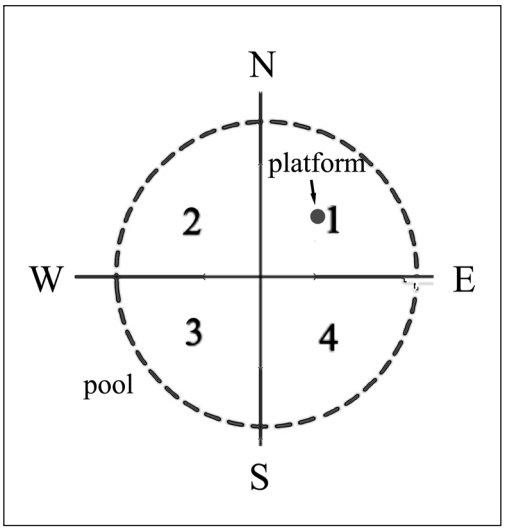

Figure 2. Diagram showing the location of the platform and the quadrants in the Morris maze task.1, 2, 3, 4 represent the quadrants of the pool.

confined in their non-preferred compartment for 45 min on days 4, 6 and 8; on days 5, 7 and 9, they were injected with saline (0.4 ml, i.p.) and confined in their preferred compartment. The dose of morphine was selected based on our previous work (Gao et al., 2012; Wu et al., 2012a, 2012b; Yonghui et al., 2006). To study the effects of AI/GI inactivation, we injected Bac-Mus mix or vehicle into the AI/GI 10–15 min prior to morphine/saline administration during conditioning. On post-test day, rats were allowed 15 min to freely move in the same apparatus to assess preference for the two compartments (Figure 1(b)).

CPA. CPA is a recognized paradigm of negative affective learning (Watanabe et al., 2003). The present CPA procedure induced aversion with acute opioid dependence, which entails naloxone-precipitated withdrawal following a dose of morphine infusion (Azar et al., 2003). This treatment can elicit a broad range of symptoms similar to those observed in a chronic opioid dependence (Azorlosa et al., 1994).

As shown in Figure 1(a), the CPA procedure included four phases: adaptation (day 1), pre-test (days 2–3), conditioning (days 4–9), and pros-test (day 10). The adaptation and pre-test phases were similar to those in the CPP procedure.

On the conditioning days, an unbiased CPA design was used, where the acute-morphine-withdrawal-paired compartment was randomly assigned for each rat. On days 4, 6 and 8, each rat was injected with naloxone (0.3 mg/kg, i.p.) 4 h after receiving morphine injection (5 mg/kg, i.p.) to induce enhanced withdrawal and confined in its acute-morphine-withdrawal-paired compartment for 45 minutes; on alternating days (5, 7 and 9), the rat was injected with saline (0.4 mL, i.p.) 4 h after receiving saline injection (0.4 mL, i.p.) and confined in its saline-paired compartment (Figure 1(c)). The dose of morphine and naloxone was selected based on our previous work (Wu et al. 2012a, 2012b).

On post-test day, each rat was placed in the same apparatus for 15 min to assess preference for the two compartments.

To confirm that the CPA seen in the present study was induced by naloxone-precipitated acute morphine withdrawal (morphine-naloxone) but not by spontaneous withdrawal after morphine administration or naloxone alone, we compared the effects of morphine-naloxone treatment on the acquisition of CPA with three control groups wherein the rats were injected with saline-saline, morphine-saline or saline-naloxone, instead of morphine-naloxone, on naloxone-conditioning days. These three control groups are referred to as the saline-saline, morphine-saline and saline-naloxone groups, respectively. All procedures were the same as in the afore-described CPA paradigm, except the treatment on naloxone-conditioning days.

To examine the effects of AI/GI inactivation on negative affective learning related to morphine-withdrawal, a separate group of rats were used and Bac-Mus mix or vehicle was injected into the AI/GI 10–15 min prior to naloxone/saline administration on the conditioning days (Figure 1(c)).

Morris water maze. It is possible that inactivation of the AI/GI affects general motor and spatial learning abilities. Therefore, we examined a new cohort of rats for mobility and spatial learning after inactivation of the AI/GI in the Morris water maze. Bac-Mus mix or vehicle was injected into the AI/GI before the first trial start on each of the training days.

The procedure was based on earlier studies with minor modification (Buccafusco and Terry, 2009; McDonald et al., 2010). The experiment consisted of 18 consecutive trials (three trials each day). The platform was hidden in a fixed position in the pool (Figure 2) throughout the experiment. At the start of a trial, a rat was placed into the water facing the pool wall and allowed to search for the platform for 60 s. The start quadrants for the first nine trials followed a pseudorandom sequence for all rats (2, 3, 4, 3, 4, 2, 4, 2, 3) and repeated for the other nine trials. Once located the platform, the rat was permitted to remain on it for 15 s. If not, it was placed on the platform for 15 s. At the end of each trial, rats were removed from the pool and placed on a dry washcloth for

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300 s before starting the next trial. Escape latency was recorded in each trial and averaged over three trials to generate escape latency of the day for a given animal.

Cannula verification

At the end of each experiment, all rats were anesthetized with chloral hydrate (400 mg/kg) and perfused transcardially with saline followed by 4.0% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). Coronal sections (40 μm) through the injection site were cut using Leica CM1900 cryostat (Leica Microsystems Nussloch GmbH, Heidelberger, Germany). The brain slices were stained according to standard Nissl-staining procedures (Meyer et al., 2008). Schematic illustrations of cannula placement are shown in Figure 3.

Statistical analysis

Time spent in the drug-paired side was analyzed using three-way repeated measures analysis of variance (ANOVA) with treatment (vehicle, inactivation) and brain area (AI, GI) as between-subject factors and test (pre-test, post-test) as a within-subject factor. Escape latency and swimming velocity were analyzed using three-way repeated measures ANOVA with treatment and brain area as between-subject factors, and days as the within-subject factor. All data are expressed as mean ±standard error of the mean (SEM) and analyzed using SPSS 13.0 software for Windows. The significant level was set at $p<0.05$.

Results

Cannula verification

Placements of infusion needle tips targeted at AI or GI were examined histologically by an observer blind to the drug treatment. Fourteen of 120 rats were removed due to incorrect placements (10 rats in Morris water maze, and 4 rats in CPP/CPA).

Effects of GI and AI inactivation on the acquisition of morphine-induced CPP

To determine whether AI/GI inactivation alters the morphine-related positive affective learning, rats receiving pre-training injection of Bac-Mus mix or vehicle into the AI/GI were trained for morphine-induced CPP (Figure 4). We observed no difference in locomotor activity between vehicle and Bac-Mus groups in AI/GI in the training and test of CPP (data not shown). Time spent in the morphine-paired side revealed a significant main effect of brain area ($F(1,34)=11.01$, $p<0.05$), treatment ($F(1,34)=5.06$, $p<0.05$) and significant interactions of treatment×brain area ($F(1,34)=6.667$, $p<0.05$) and of treatment×brain area×test ($F(1,34)=5.579$, $p<0.05$). Bonferroni post-hoc analysis indicated that GI groups showed a significant difference in the post-test phase ($p<0.05$), but not in the pre-test phase ($p>0.05$). That is, the GI inactivation group ($n=11$) spent less time in the morphine-paired side than did the control group ($n=11$) ($p<0.05$) in the post-test phase. On the contrary, AI groups showed no significant difference in the pre-test phase ($p>0.05$), or in the post-test phase ($p>0.05$). These results suggested that AI/GI inactivation had different effects on the acquisition of morphine-induced CPP: the GI rather than the AI was involved in the acquisition of morphine-related positive affective learning.

Effects of GI and AI inactivation on the acquisition of naloxone-precipitated CPA

To confirm that acute morphine withdrawal is necessary and sufficient to induce a CPA in the current paradigm, we compared the effects of morphine-naloxone, morphine-saline and saline-naloxone on the acquisition of CPA without manipulation of insular subdivisions. Time spent in drug-paired side revealed a significant main effect of group ($F(3,28)=3.46$, $p<0.05$), test ($F(1,28)=4.742$, $p<0.05$) and a significant interaction of group×test ($F(3,28)=4.573$, $p<0.05$). Bonferroni post-hoc analysis found that the morphine-naloxone group spent significantly less time in the drug-paired side than the saline-saline control group in the post-test ($p<0.001$), but this was not found for the morphine-saline or saline-naloxone groups (Figure 5). Therefore, the CPA seen in the present study was induced by naloxone-precipitated acute morphine withdrawal but not by spontaneous withdrawal after morphine administration or naloxone alone.

To determine whether AI/GI inactivation alters the opiate-related negative affective learning, rats receiving pre-training injection of Bac-Mus mix or vehicle into the AI/GI were trained for naloxone-precipitated CPA (Figure 6). Time spent in the naxolone-paired side revealed a significant main effect of treatment ($F(1,32)=4.369$, $p<0.05$), but not that of brain area ($F(1,32)=0.605$, $p>0.05$); we observed no significant interaction of treatment×brain area ($F(1,32)=0.183$, $p>0.05$) or that of treatment×brain area×test ($F(1,32)=0.141$, $p>0.05$). That is, injecting Bac-Mus mix in both the GI and in the AI prior to conditioning attenuated the acquistion of naloxone-precipitated CPA. The results suggested that AI and GI inactivation similarly affected the acquisition of naloxone-precipitated CPA: both of the two subdivisions were involved in the acquisition of opiate-related negative learning.

Effects of GI and AI inactivation on the learning and motor ability in the Morris maze task

A new cohort of rats with pre-training inactivation of the AI/GI were trained using the Morris water maze task to detect possible alteration of general motor and spatial learning ability, as indexed by swimming velocity and escape latency (Figure 7).

The escape latency showed a significant main effect of day ($F(5,130)=45.835$, $p<0.001$), but no significant main effect of treatment ($F(1,26)=1.414$, $p>0.05$), brain area ($F(1,26)=1.611$, $p>0.05$) or significant interaction of day×brain area ($F(5,130)=0.709$, $p>0.05$), day×treatment ($F(5,130)=0.551$, $p>0.05$) or treatment×brain area ($F(1,26)=0.950$, $p>0.05$). In the analysis on swimming velocity, we observed a significant main effect of day ($F(5,130)=3.7$, $p<0.05$) and brain area ($F(1,26)=9.317$, $p<0.05$), but not that of treatment ($F(1,26)=0.722$, $p>0.05$). However, we did not find interaction of treatment×brain area ($F(1,26)=0.529$, $p>0.05$). As shown in Figure 7(b), AI groups swam slower than GI groups regardless of inactivation treatment. These results suggested that neither AI

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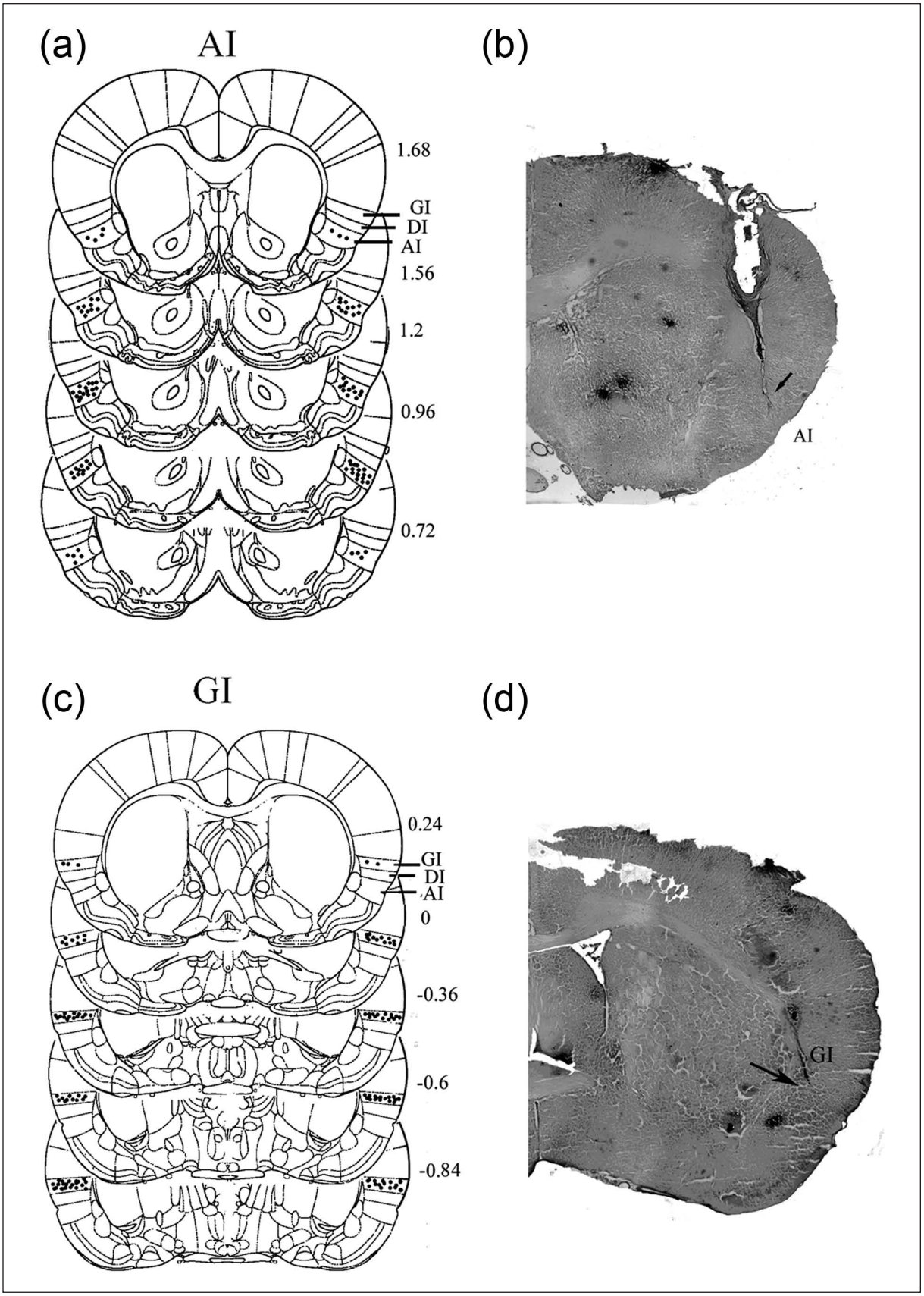

Figure 3. Schematic illustrations and representative photomicrographs of the intracranial cannula infusion sites in agranular insula (a and b) and granular insula (c and d). Black dots show locations of injector tips for the rats included in statistical analysis. Numbers on the right indicates the the distance posterior to bregma. The figure is adapted from diagrams of a stereotaxic atlas of the rats brain (adapted from Paxinos G and Watson C (2005) *The Rat Brain in Stereotaxic Coordinates*, 5th ed. with permission from Elsevier). AI: agranular insula; DI: dysgranular insula: GI: granular insula.

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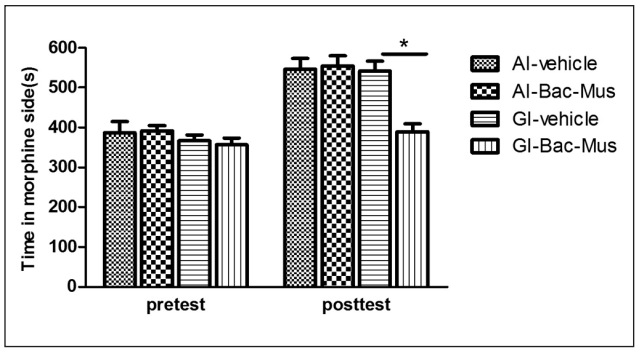

Figure 4. Effects of agranular insula (AI) and granular insula (GI) inactivation on the acquisition of morphine-induced conditioned place preference (CPP).
Data are expressed as means±standard error of the mean (SEM). AI-vehicle n=8, AI-Bac-Mus n=8, GI-vehicle n=11, GI-Bac-Mus n=11; *p<0.05. Bac-Mus: baclofen-muscimol mixture.

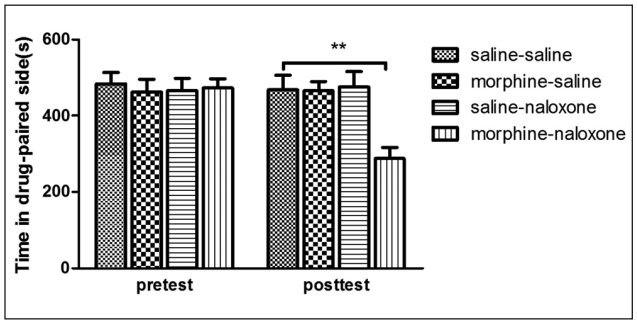

Figure 5. Effects of morphine-naloxone, morphine-saline and saline-naloxone on the acquisition of conditioned place aversion (CPA).
Data are expressed as means±standard error of the mean (SEM), n=8 for all groups, **p<0.001.

nor GI inactivation impaired the general spatial learning or motor ability.

Discussion

The main findings of the present study are: (a) inactivation of the GI but not that of the AI attenuated the acquisition of morphine-induced CPP; (b) both AI and GI inactivation attenuated the acquisition of naloxone-precipitated CPA; (c) neither AI nor GI inactivation had effects on the spatial learning or motor ability in Morris water maze. These data suggested that the AI and the GI differentially regulate positive and negative opiate-related affective learning and that the effects are specifically on affective learning but not on general mobility or learning ability.

The interoceptive system, particularly the insular cortex, has attracted increasing attention in studies of drug addiction (Contreras et al., 2007, 2012; Forget et al., 2010; Hollander et al., 2008; Naqvi et al., 2007) because it represents the bodily changes underlying the reinforcing properties of drugs and is highly plastic to recent experiences (Accolla and Carleton, 2008; Craig, 2002; Damasio et al., 2000). The insular cortex has been implicated in many phases of the development of drug addiction, for

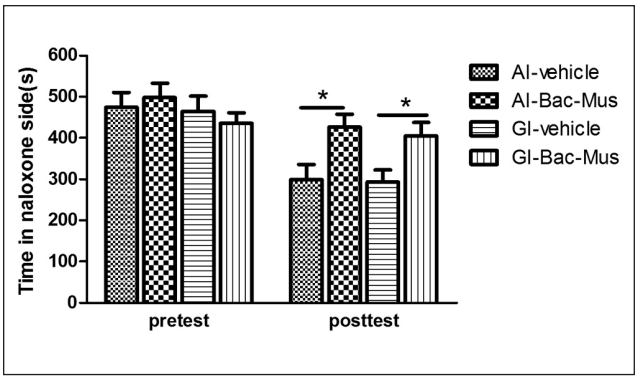

Figure 6. Effects of insular inactivation on the acquisition of naloxone-precipitated conditioned place aversion (CPA).
Data are expressed as means±standard error of the mean (SEM), AI-vehicle n=7, AI-Bac-Mus n=9, GI-vehicle n=10, GI-Bac-Mus n=10. *p<0.05. Bac-Mus: baclofen-muscimol mixture.

example, the expression (Contreras et al., 2007), extinction, reconsolidation (Contreras et al., 2012) and the reinstatement (Forget et al., 2010) of nicotine/amphetamine seeking behavior. Yet, few studies have addressed the role of the insular cortex and its functional heterogeneity on the acquisition of opiate-related affective learning.

Neural connections of the insula suggest that the AI is a better candidate than the GI as a hub where the opiate-related affective learning takes place. The GI mainly receives visceral inputs from the thalamus and parabrachial nuclei (Allen et al., 1991), whereas the AI is interconnected with the regions representing spatial context (Insausti et al., 1997; Kerr et al., 2007) and the regions representing the reinforcing properties of drugs of abuse (Allen et al., 1991; Cechetto, 1987; Chikama et al. 1997; Ohara et al., 2003). Therefore, the AI rather than the GI may be the insula subdivision where the opiate-related affective learning takes place.

However, our results did not support the afore-mentioned hypothesis. We found that inactivation of the AI only blocked the acquisition of naloxone-precipitated CPA, but not that of morphine-induced CPP. One possible explanation is that the morphine-induced primary sensory changes are not relayed to the AI. This is unlikely because lesions of the AI attenuated the acquisition of morphine-induced CTA (Lin et al., 2009; Mackey et al., 1986). Another likely possibility is that the AI integrates only negative affect with morphine- and withdrawal-induced sensory changes. Indeed, the AI receives projections from the amygdala and the medial thalamic nuclei which are both associated with negative affect (Allen et al., 1991, Jasmin et al., 2004).

On the other hand, inactivation of the GI attenuated the acquisition of both morphine-induced CPP and naloxone-precipitated CPA, consistent with the mounting evidence suggesting a critical role of the GI in drug-related affective processes. For example, blockade of hypocretin transmission in the GI abolished the rewarding effects of nicotine (Hollander et al., 2008). The role of the GI on the acquisition of opiate-related affective learning is usually attributed to its sensory function (Contreras et al., 2007). Our results suggested that the visceral sensory information might be a critical element of the reinforcing effects of opiates/opiate

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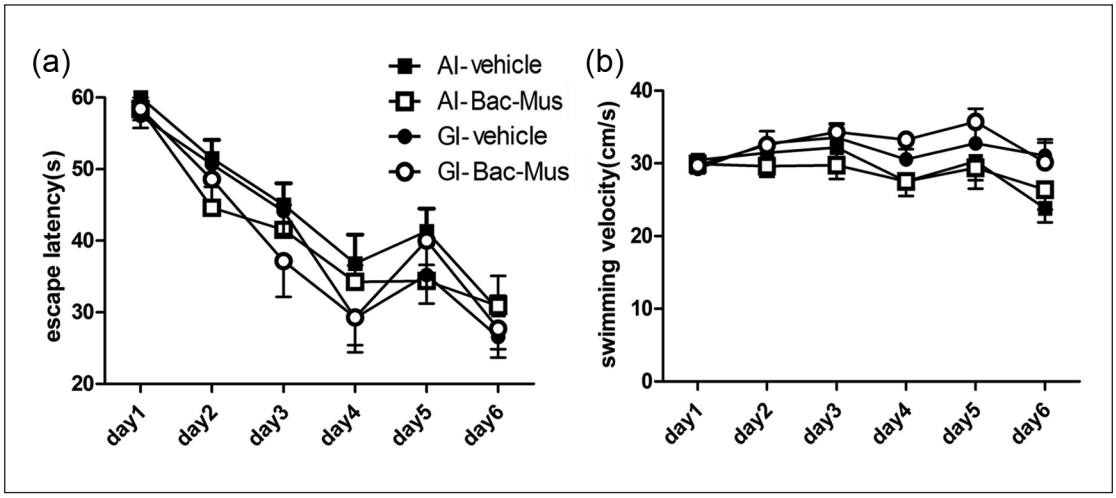

Figure 7. Effects of agranular insula (AI) and granular insula (GI) inactivation on (a) the learning and (b) swimming velocity of rats in the Morris maze task.

Data are expressed as means±standard error of the mean (SEM). AI-vehicle *n*=6, AI-Bac-Mus *n*=8, GI-vehicle *n*=9, GI-Bac-Mus *n*=7. Bac-Mus: baclofen-muscimol mixture.

withdrawal. These data supported the somatic marker theory of addiction, which proposes that the special feelings generated from drug use/withdrawal can be connected with anticipated future outcomes of certain scenarios by learning (Verdejo-Garca and Bechara, 2009). Therefore, the extinction of visceral response induced by drug-related cues might be very important in treating opiate addiction.

Moreover, recent evidence suggested that the insular cortex, including the GI, might also directly mediate the reinforcing properties of drugs of abuse. Electrical stimulation of the insular cortex induced flavor preferences in rats (Cubero and Puerto, 2000); furthermore, blockade of hypocretin transmission in the GI abolished the rewarding effects of nicotine indicated by reversal of nicotine-induced lowering of intracranial self-stimulation thresholds (Hollander et al., 2008). Further studies on the insular regulation of drug reinforcement are desirable.

In conclusion, the present study demonstrated that the insula mediates opiate-related positive and negative affective learning, at least at the initial stages of addiction. Moreover, the GI appears critical for both forms of affective learning, whereas the AI is crucial for the negative affective learning associated with opiate withdrawal. Furthermore, it is widely accepted that initial drug use is mainly motivated by the positive affective consequence of opiates: whereas, after periods of abstention, drug use may be motivated mainly by the negative affective effects induced by opiate withdrawal (Schulteis and Koob, 1996). Hence, the present data suggested that the GI rather than the AI plays an important role in initial drug use and that both the GI and the AI are crucial for continued drug use after periods of abstention. Further studies are required to address this issue.

Conflict of interest

The authors declare that there are no conflicts of interest.

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