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DAMGO in the central amygdala alleviates the affective dimension of pain in a rat model of inflammatory hyperalgesia

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Abstract

Pain has sensory-discriminative and emotional-affective dimensions. Recent studies show that the affective component can be assessed with a conditioned place avoidance (CPA) test. We hypothesized that systemic morphine before a post-conditioning test would more potently attenuate the affective aspect compared to the sensory component and that DAMGO, a μ -selective opioid receptor agonist, injected into the central nucleus of the amygdala (CeA) would reduce established CPA. A rat model of inflammatory pain, produced by a complete Freund adjuvant (CFA) injection into the hind paw, was combined with a CPA test. Three experiments were performed on adult male Sprague-Dawley rats. Systemic morphine (0.5 or 1.0 mg/kg) in Experiment 1, intrathecal (i.t.) morphine (2.5 μ g/rat) in Experiment 2, and intra-CeA DAMGO (7.7-15.4 ng/0.4 μ l) in Experiment 3 were given to CFA-injected rats (n=6-8/group) prior to a post-conditioning test. Saline-injected rats were used as control. Time spent in a pain-paired compartment was recorded twice, before conditioning and after a post-conditioning test. Paw withdrawal latency (PWL) to a noxious thermal stimulus was measured before experiment at day -1 and after the post-conditioning test; hyperalgesia was defined as a decrease in PWL. The data showed that CFA-injected rats had significantly negative CPA compared to those of saline-injected rats ($P<0.05$). Low dosage systemic morphine significantly ($P<0.05$) reduced CFA-induced CPA but had no effect on PWL. I.t. morphine did not inhibit the display of CPA but significantly increased PWL, suppressing hyperalgesia ($P<0.05$). Intra-CeA DAMGO significantly inhibited the display of CPA compared to saline ($P<0.05$) but had no effect on PWL. The data demonstrates that morphine attenuates the affective component more powerfully than it does the sensory and suggests that the sensory and the emotional-affective dimensions are underpinned by different mechanisms.

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AUTHOR CONTRIBUTIONS R.X.Z., M.Z., A. L., and L. P. performed surgeries and behavioral and histological experiments. R.X.Z., K.R., and L.L. designed the experiments, analyzed data, and contributed to the writing of the manuscript. B.M.B. contributed to the writing of the manuscript.

Conflicts of interest None.

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Keywords

Conditioned place avoidance; pain; opioid; amygdala; spinal cord; hyperalgesia

1. Introduction

The experience of pain has both sensory-discriminative and emotional-affective dimensions. Recent studies showed that the affective component can be assessed with a conditioned place avoidance (CPA) test or place avoidance test (Johansen et al., 2001, King et al., 2009, LaBuda and Fuchs, 2000, Zhang Y et al., 2011). It has been reported that lower dosages of morphine reduced the affective aspect of pain but not its sensory aspect in a clinical setting (Price et al., 1985, Kupers et al., 1991, Jensen, 1997), suggesting that morphine more potently attenuates the former. Consistent with those findings, low dosage morphine alleviated affective pain but not mechanical allodynia in a neuropathic pain rat model (LaGraize et al., 2006). We hypothesized that systemic morphine administration prior to a post-conditioning test would more potently attenuate the affective component in an inflammatory pain rat model.

The neural systems of the sensory component of pain have been extensively studied, but only a few studies have investigated the central mechanisms of the affective component. One such study showed that morphine administration into the anterior cingulate cortex (ACC) decreased pain affect but not mechanical allodynia, suggesting ACC involvement in the former (LaGraize et al., 2006). Investigators also reported that intra-spinal pain medicine before pain-paired conditioning blocked the acquisition of CPA (King et al., 2009), but there had been no investigation of the effect of intra-spinal pain medicine on the display of established CPA. We hypothesized that spinal morphine would not reduce established CPA behavior.

Previous studies have shown that the amygdala is an important supraspinal-level nucleus for the regulation of emotional behavior (LeDoux, 2000). Excitotoxic lesion of the central amygdala (CeA) significantly reduced the magnitude of intra-pantar formalin-induced CPA but did not alter acute formalin-induced nociceptive behaviors (Tanimoto et al., 2003, Gao et al., 2004). The data demonstrate that the CeA is involved in CPA modulation. It has also been shown that the CeA contains opioid receptors which can be activated with the μ receptor agonist (DAMGO) [D-Ala2-N-Me-Phe4, Gly-ol5]-enkephalin (Ding et al., 1996, Daunais et al., 2001). Taken together, these data suggest that opioid receptors in the CeA might be involved in systemic, morphine-produced inhibition of CPA. We hypothesized that intra-CeA administration of DAMGO prior to a post-conditioning test would inhibit CPA behavior, and we investigated the hypothesis with a CFA-induced inflammatory pain rat model combined with a CPA test (Zhang Y et al., 2011).

2. Experimental Procedures

2.1. Animal Preparation

Male Sprague-Dawley rats weighing 250–275 g (Harlan, Indianapolis, IN) were kept under controlled laboratory conditions (22°C, relative humidity 40%–60%, 12-hour alternate light–dark cycles, food and water *ad libitum*) and were acclimatized to the environment for five days prior to experimentation. The animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Maryland School of Medicine, Maryland, USA.

2.2. Experimental Design

Three experiments were conducted.

In Experiment 1, to investigate the effect of systemic non-analgesic dosages (0.5 and 1 mg/kg) of morphine on CFA-induced negative affective response, four groups of rats were given intraperitoneal (i.p.) morphine or saline prior to a post-conditioning test ($n=6/\text{group}$): **1)** Intra-hind paw saline, **2)** intra-hind paw saline plus morphine at 1 mg/kg (i.p.), **3)** intra-hind paw CFA plus saline (0.25 ml, i.p.), **4)** CFA plus morphine at 0.5 mg/kg in 0.25 ml saline, and **5)** CFA plus morphine at 1 mg/kg. Saline (0.08 ml) or CFA (Sigma, St Louis, MO; 0.08 ml, 40 μg *Mycobacterium tuberculosis*), suspended in an 1:1 oil/saline emulsion, was injected into one hind paw 30 min after non-CFA paired conditioning, that is, 2 h before CFA-paired conditioning (Zhang Y et al., 2011). Saline (0.25 ml, i.p.) or morphine was administered 30 min before the post-conditioning test on the third day (Fig. 1).

In Experiment 2, to investigate the effect of intrathecal (i.t.) morphine (2.5 μg , Sigma) on CFA-induced negative affective response, rats were divided into four groups ($n = 8/\text{group}$): **1)** Intra-hind paw saline, **2)** intra-hind paw CFA, **3)** CFA + morphine (i.t., 2.5 μg in 5 μl), and **4)** CFA + saline (i.t. 5 μl). Morphine was given to rats 30 min prior to a post-conditioning test on day three to investigate its effect on CFA-induced CPA.

In Experiment 3, to investigate the involvement of CeA opioids in the modulation of CPA, rats were prepared for bilateral cannulation into the CeA or the basolateral amygdaloid nucleus (BLA) and allowed to recover for five days prior to experiment. Rats with intra-CeA cannulation were divided into five groups ($n = 7/\text{group}$): **1)** intra-paw saline + DAMGO (15.4 ng in 0.4 μl per side), **2)** intra-paw saline + saline, **3)** intra-paw CFA + DAMGO (7.7 ng in 0.4 μl per side), **4)** CFA + DAMGO (15.4 ng in 0.4 μl per side), and **5)** CFA + saline. DAMGO at 15.4 ng in 0.4 μl per side was infused into the BLA in another 9 CFA-injected rats used as site-specific control. DAMGO was dissolved in saline and infused into the CeA or BLA 20 min before a post-conditioning test on day three. The antagonist dosages are based on our preliminary study. Paw withdrawal latency (PWL) to a noxious thermal stimulus was measured after a post-conditioning test to determine whether DAMGO attenuates thermal hyperalgesia.

2.3. CPA Test

The CPA test was performed as previously reported (Zhang Y et al., 2011) using a place conditioning apparatus made of Plexiglas. It consists of two equal rectangular compartments, 12" long $\times$ 8" high $\times$ 5" wide, positioned on mesh screen floors and separated by guillotine doors. There is no neutral third chamber in the center. One compartment is covered on three sides and the ceiling with horizontal yellow 0.5-inch stripes at 0.5-inch intervals; its bottom is #8 $\times$ 8 (8 mm holes) plain steel mesh. The other compartment is covered with vertical orange 0.5-inch stripes; its bottom is #3 $\times$ 3 (3 mm holes) plain steel mesh; a Coast Led Lenser mini-Tac Torch illuminates its exterior. The guillotine doors, covered with colored stripes corresponding to their respective walls, were inserted during conditioning sessions and removed during pre- and post-conditioning tests. The colored horizontal and vertical stripes and the light are the visual cues; the differing steel mesh is the tactile cue. The testing room was illuminated with a 15-W bulb positioned about 1 m from the apparatus. The apparatus was cleaned with 75% ethanol after each test.

On day one, baseline time spent by the rats during a 10-min preconditioning period in each of the two distinctive compartments was recorded. The animal was considered to be in a chamber when both its front paws were in it. On day two, each rat was free to explore one of the conditioning compartments for 30 min. Thirty min later, saline or CFA was

subcutaneously injected into the plantar surface of one hind paw (Zhang et al., 2004). CFA-injected rats showed a significant decrease in PWL to a noxious thermal stimulus 30 min post-CFA injection and reached peak level at 2 h (Lao et al., 2004). Two h after the CFA injection, the rat was free to explore the second compartment for another 30 min. The pain-paired compartment was assigned randomly before baseline measurement, and the compartments were counterbalanced among the subjects. For example, in experiment 1, group 3, intra-hind paw CFA plus saline rats numbers 1, 3, and 5, were pain-conditioned in a compartment with horizontal stripes while rats 2, 4, and 6 were pain-conditioned in a compartment with vertical stripes. During the 10-min post-conditioning test on day three, the time spent by the rats in each compartment was recorded again (Fig. 1). The investigator who performed the CPA test was blinded to the group assignment.

2.4. Hyperalgesia Testing

Hyperalgesia was determined by a decrease in PWL to a noxious thermal stimulus. PWL was tested with Hargreaves's method (Hargreaves et al., 1988, Lao et al., 2004) using a Paw Thermal Stimulator System (UCSD, San Diego). Four tests with a 5-min interval between each were conducted for each hind paw. Mean PWL was established by averaging the values of each test.

2.5. I.t. Morphine Delivery

Lumbar punctures were performed as previously described (Li et al., 2011). The injection catheter was pre-filled with 5 μ l of drug or vehicle and 5 μ l of saline separated by a small air bubble. Under isoflurane anesthesia, the dorsal pelvic area was shaved and swabbed with 70% alcohol. A 21-gauge sterile needle with the plastic hub removed was inserted between lumbar vertebrae L5 and L6. The catheter was inserted into the guide needle and rostrally advanced 4 cm from the tip of the needle into the lumbar enlargement, where its arrival was confirmed by a tail-flick. The drug, or vehicle, was injected and followed by a saline flush. Two min after injection the catheter was withdrawn and the needle was removed from the intervertebral space.

2.6. Intra-Bilateral CeA Cannulation

Animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and held in a stereotaxic frame (Stoelting, Wood Dale IL). As previously reported (Zhang Y et al., 2011), a 23-gauge stainless steel guide cannula (Plastic One, Roanoke, VA) was implanted toward the CeA -2.3 mm rostrally, 3.8 mm laterally, and 6 mm ventrally or the BLA -2.3 mm rostrally, 4.7 mm laterally, and 6 mm ventrally toward the bregma according to the Paxinos and Watson flat skull coordinate system. Following cannulation, animals were housed singly and allowed to recover for five days prior to the experiment.

For drug infusion, a 0.6 cm length of PE-50 tubing was connected to each end of a 15 -cm length of PE-10 tubing. During infusion, the dummy cannula was replaced by an injector that was inserted 2.0 mm or 2.5 mm beyond the guide cannula to target the CeA or the BLA. One end of the tubing was connected to the injector and the other to a 50 - μ l Hamilton syringe. The solution was infused with a pump at 0.2 μ l/min for a total of 0.4 μ l on each side of the CeA (KD Scientific, Model 780210, Holliston, MA). After infusion, the injector was left in the cannula for another 2 min to allow the chemicals to spread at the injected area.

2.7. Histology

After the experiment, the infusion site was verified by histology. The animals were perfused with saline and 10% formalin under analgesia with sodium pentobarbital. The brains were removed and immersed in 10% formalin for 2 h and transferred to 30% sucrose. The tissue

at the cannula site was cut at 40- μ m-thick coronal sections which were stained and examined under a microscope to determine the location of the cannula according to Paxinos and Watson's atlas.

2.8. Statistical Analysis

The data (mean $\pm$ SEM) were analyzed with ANOVA to reveal whether morphine or DAMGO inhibited CPA. Bonferroni post-tests were conducted to reveal differences among groups of rats (GraphPad Prism 5.0). $P < 0.05$ was considered significant.

3. Results

3.1. Systemic Non-Analgesic Morphine Reduced CFA-Induced CPA

As shown in Fig. 2A, all rats spent about 5 min in the pain-paired chambers during the 10 min pre-conditioning test, demonstrating no preference for either. This result is similar to that of our previous report (Zhang Y et al., 2012). Rats receiving CFA and i.p. saline spent significantly less time in the pain-paired compartment during the post-conditioning test than during the pre-conditioning test. This demonstrates that CFA-injected rats showed place aversion to the pain-paired compartment. By contrast, the saline-injected rats showed no aversion to the saline-paired compartment. These results establish that the CFA injection produced CPA.

Low dosage (0.5-1 mg/kg, i.p.) systemic morphine dose-dependently reduced CFA-induced CPA (Fig. 2A) but had no effect on PWL (Fig. 2B). This indicates that systemic morphine more easily attenuates the affective than the sensory component of pain. It should be noted that intra-hind paw saline plus morphine at 1 mg/kg (i.p.) in naive rats does not induce conditioned place preference. This indicates that the disappearance of CFA-induced CPA in morphine-treated rats was not due to morphine-induced preference.

3.2. I.t. Morphine Did Not Inhibit CFA-Induced CPA

CFA induced CPA but saline did not (Fig. 3A). CFA-injected rats given i.t. saline or morphine (2.5 μ g/rat) spent less time in the pain-paired compartment after conditioning than before. This indicates that the CFA injection induced an aversion to the pain-paired compartment during the post-conditioning test and that the spinal morphine did not reduce established CPA (Fig. 3A).

As shown in Fig. 3B, morphine at 2.5 μ g significantly increased PWL to a noxious thermal stimulation.

3.3. An Infusion of DAMGO into the CeA Inhibited CFA-Induced CPA

We injected DAMGO or saline into the CeA of saline- or CFA-injected rats. As shown in Fig. 4A, intra-paw CFA plus intra-CeA saline rats spent significantly less time in the pain-paired compartment during the post-conditioning test than during the preconditioning test, showing place avoidance to the pain-paired compartment. In contrast, CFA plus intra-CeA DAMGO showed no aversion to that chamber. These results suggest that μ opioid receptor activation in the CeA is involved in the inhibition of CPA. Intrapaw saline plus intra-CeA saline or DAMGO produced no avoidance or preference behavior, demonstrating that DAMGO activation of μ opioid receptors in the CeA does not affect such behavior. A bilateral infusion of 15.4 ng of DAMGO into the BLA did not significantly modulate CFA-induced CPA, suggesting that BLA μ opioid receptors are not involved in CPA behavior. DAMGO administration into the CeA or the BLA did not affect PWL (Fig. 4B).

3.4. Histology

Cannula and injection sites were located in the CeA or BLA as shown in Fig. 5.

4. Discussion

4.1. Systemic Morphine and CPA

In the present study, a CFA injection induced an avoidance response to a pain-paired compartment compared to a saline injection, indicating that the CFA produced affective pain. This is consistent with previous reports that a formalin (Johansen et al., 2001) or a carrageen (van der Kam et al., 2008) injection induced affective pain. In the present study, systemic, low-dosage (0.5-1 mg/kg, i.p.) morphine prior to a post-conditioning test significantly inhibited the display of CPA but did not increase PWL. In our previous study in the same CFA-induced inflammatory pain model, morphine at 5-7.5 but not 2.5 mg/kg significantly increased PWL, demonstrating anti-hyperalgesia (Zhang et al., 2004). Consistent with these results, previous studies using escape/avoidance testing showed that low-dosage celecoxib, diclofenac, or duloxetine given before the tests significantly inhibited the inflammatory pain-induced avoidance response, whereas higher dosages of these drugs were needed to attenuate mechanical allodynia (Boyce-Rustay et al., 2010). Similarly, in a neuropathic pain rat model, studies used escape/avoidance testing to show that low-dosage morphine attenuates place escape/avoidance behavior but not mechanical hyperalgesia (LaGraize et al., 2006). Thus low-dosage analgesics attenuated the affective but not the sensory aspect of pain in both inflammatory and neuropathic pain models to show that morphine has a more potent effect on affective than on sensory pain.

CPA depends on the association of spontaneous pain-induced affect with a particular environmental context. There is a possibility that morphine interfered with the rat's memory of this relationship, thus confounding interpretation of the data. However, it has been reported that morphine at 5.6mg/kg did not impair performance in a nose-poke repeated performance test (Pitts et al., 2006), and pre-test subcutaneous administration of 5 mg/kg morphine had no effect on memory retrieval in a step-down avoidance test (Lu et al., 2010). Further, post-training administration of 2.5 mg/kg morphine did not significantly reduce step-through latency in a step-through passive avoidance study (Khajepour et al., 2008). These data suggest that the morphine used in the present study did not interfere with the rats' associational memory. The morphine inhibition of CPA was also not the result of a reward effect because intra-hind paw saline plus morphine at 1 mg/kg (i.p.) in naive rats does not induce conditioned place preference.

4.2. The Effect of I.t. Morphine on CPA

In a previous study, spinal administration of the α_2 adrenergic agonist clonidine prior to pain-paired conditioning increased the time rats spent in a clonidine-paired chamber, with a corresponding decrease in time spent in the saline-paired chamber (King et al., 2009). The data demonstrate that ascending noxious messages from the spinal cord are involved in the acquisition of affective pain. This is consistent with our unpublished data showing that i.t. analgesic morphine before pain-paired conditioning prevented CPA behavior, which indicated that the drug blocked the acquisition of affective pain. In the present study, i.t. analgesic morphine after pain-paired conditioning and before a post-conditioning test did not abolish the affective pain. These data suggest that the spinal processing of noxious messages does not play a critical role in the maintenance of affective pain although it does in its acquisition.

4.3. The Effect of Intra-CeA DAMGO on CPA

Since spinal morphine has no effect on the display of affective response, the systemic morphine data indicates that supraspinal nuclei are involved in the maintenance of affective pain. This is substantiated by our study demonstrating that intra-CeA DAMGO (7.7-15.4 ng per side) prior to a post-conditioning test significantly inhibited CPA. In previous studies, excitotoxic lesions of the CeA abolished formalin-induced CPA (Tanimoto et al., 2003, Gao et al., 2004). Further, it has been shown that intra-CeA N-methyl-D-aspartate (NMDA) or non-NMDA receptor antagonist inhibited vocalizations, an indication of pain affect, induced by brief noxious tail shock, but not spinal motor reflexes (Spuz and Borszcz, 2012). These data indicate that the CeA is involved in the maintenance of affective pain. On the other hand, our study showed that intra-CeA DAMGO at 7.7-15.4 did not increase PWL. In previous studies, intravenous morphine significantly suppressed CeA neural response to noxious stimuli, suggesting that the CeA is involved in morphine inhibition of pain (Huang et al., 1993). Further studies showed that neurotoxic lesions of the CeA attenuated morphine inhibition of nociceptive behavior such as flinching in a formalin-induced pain model (Manning and Mayer, 1995, Manning, 1998) and that intra-CeA morphine at 50-100 μ g significantly produced naloxone-reversible inhibition of formalin-induced nociceptive behavior (Sabetkasaei et al., 2007). Collectively, these studies lead to the hypothesis that low and moderate activation of CeA μ opioid receptors might be involved in the inhibition of pain affect while high activation might also be involved in the inhibition of the sensory component of pain.

In the present study, we also found that intra-BLA DAMGO did not attenuate the CFA-induced CPA, suggesting that BLA opioids are not involved in maintaining affective pain. In support of this, it has been reported that i.p. acetic acid-induced CPA was abolished by CeA but not BLA lesions. These data suggest that CeA and BLA play different roles in specific conditions such as CPA maintenance. It should be noted that an intra-BLA injection of morphine given before pain-paired conditioning in a dosage that does not affect the sensory component of pain attenuated formalin-induced negative affective response (Deyama et al., 2007), which suggests that BLA opioids are involved in the acquisition of affective response. These data suggest that BLA opioids play different roles during acquisition and display of pain-induced affective response. However, we do not exclude the possibility that the difference might be accounted for by the use of different animal models or CFA instead of formalin.

5. Conclusions

In conclusion, the present study demonstrates that morphine more powerfully attenuates the affective component than it does the sensory aspect of pain, that spinal processing of noxious messages is not involved in the maintenance of affective pain, and that CeA opioids are involved in the maintenance of such pain.

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List of abbreviations

BLA	basolateral amygdaloid nucleus
CeA	central nucleus of the amygdala

CFA	complete Freund adjuvant
CPA	conditioned place avoidance
DAMGO	[D-Ala ² -N-Me-Phe ⁴ , Gly-ol ⁵]-enkephalin
IACUC	Institutional Animal Care and Use Committee
NMDA	N-methyl-D-aspartate
PWL	Paw withdrawal latency

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- CFA injection into hindpaw induced significant conditioned place avoidance (CPA).
- Low doses of systemic morphine inhibited CFA-induced CPA but had no effect on PWL.
- I.t. morphine did not inhibit the display of CPA but significantly increased the PWL.
- Intra-CeA DAMGO significantly inhibited the CPA but had no effect on the PWL.
- The sensory and the affective dimensions are underpinned by different mechanisms.

Fig. 1.

Flow chart of the experimental design. On day one, time spent by rats in each of two distinct compartments was recorded during a 10-min preconditioning period. On day two, each rat freely explored the first compartment for 30 min before a CFA injection and the second compartment for 30 min after CFA. On day three, after morphine treatment, the time spent in each compartment during a 10-min post-conditioning test was recorded again. PWL was measured after the CPA test.

Fig. 2.

A. Effects of a systemic non-analgesic dosage (0.5, 1 mg/kg, i.p.) of morphine on affective pain (n=6/group). Saline-injected rats spent equal time in the saline-paired compartment during pre- and post-conditioning tests. CFA-injected rats spent less time in the pain-paired compartment during post-conditioning test than during the preconditioning test, demonstrating a CPA response to the pain-paired compartment. Morphine at a non-analgesic dosage significantly abolished CFA-induced aversive response when given i.p. before a post-conditioning test. * $P < 0.05$ vs. pre-conditioning test. **2B.** Effects of systemic morphine (1 mg/kg, i.p.) on hyperalgesia (n=6/group). Compared to saline control, morphine at 1 mg/kg did not increase PWL to a noxious thermal stimulation.

Fig. 3.

A. Effects of i.t. morphine (2.5 μ g / rat) on CFA-induced affective pain (n=6/ group). Saline-injected rats spent equal time in the saline-paired compartment during pre- and post-conditioning tests. CFA-injected rats spent less time in the pain-paired compartment during the post-conditioning test than during the pre-conditioning test, demonstrating a CPA response to the pain-paired compartment. I.t. saline or morphine treatment prior to a post-conditioning test did not block the aversive response. *P<0.05 vs. the preconditioning test.

3B. Effect of i.t. morphine on PWL of the CFA-injected paw (n=6/ group). Morphine at 2.5 μ g/5 μ l, given 30 min before the post-conditioning test, significantly increased PWL compared to saline control, which shows that morphine inhibited the CFA-induced thermal hyperalgesia. *P<0.05 vs. saline control.

Fig. 4.

A. Effects of μ opioid receptor agonist DAMGO on CFA-induced affective response ($n=7/$ group). DAMGO or saline was infused into the CeA 20 min prior to a post-conditioning test in CFA- and saline-injected rats. DAMGO significantly attenuated aversive response in CFA-injected rats compared to saline control. Saline-injected rats showed no place avoidance when given either DAMGO or saline. DAMGO infused into the BLA did not attenuate CFA-induced aversive response. $*P<0.05$ vs. the preconditioning test. **4B.** Effect of intra- CeA or BLA DAMGO on PWL of the CFA-injected paw ($n=7/$ group). DAMGO at 7.7-15.4 ng in the CeA and 15.4 ng in the BLA did not significantly increase PWL compared to saline control. $*P<0.05$ vs. saline-injected rat control.

Fig. 5.
A. A representative microphotograph of an injection site in the CeA. **5 B.** Histological verification of cannula placement in the CeA and BLA. Distribution of DAMGO or saline microinjection sites in the CeA and BLA are shown in the graphs. Each symbol represents one rat in the corresponding group. The rostral-to-caudal coordinate, -2.3 mm, is with respect to the bregma.