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Corticofugal outputs facilitate acute, but inhibit chronic pain in rats

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ABSTRACT

It has been widely accepted that the primary somatosensory cortex (SI) plays an essential role in the sensory-discriminative aspect of pain perception. However, it remains unclear whether the SI has a role in the descending modulation of pain. Although there are abundant fibers projecting back from sensory cortex to thalamic nuclei, and the influence of cortical modulation from SI on the thalamic nociceptive relay neurons has been addressed, little is known about how the cortical outputs modulate the nociceptive behaviors resulting from tissue injury or evoked by painful stimulation. The present study was designed to test whether the cortical outputs influenced the nociceptive behaviors using rat models of noxious thermal-induced acute pain, formalin-induced acute and CFA-evoked chronic inflammatory pain. The results showed that intracortical microinjection of $GABA_A$ agonist muscimol significantly reduced the first and second phase behaviors in formalin tests and elevated the nociceptive thresholds in the thermal stimulus-elicited acute pain, suggesting a facilitatory influence of SI on the acute pain sensation. By contrast, microinjection of $GABA_A$ antagonist bicuculline remarkably reduced the thermal hyperalgesia of the CFA-inflamed hindpaws, indicating an inhibitory effect of SI output in the chronic pain state. The opposite modulatory effects in acute and chronic pain states suggest that there exists a functional switch for the SI cortex at different stages of pain disease, which is of great significance for the biological adaptation.

1. Introduction

The nociceptive system is composed of transduction, transmission, modulation and perception of pain [36,48]. Evidence exists that the central nociceptive processing, like that of other kinds of sensation, involves not only afferent, but also efferent neural activity [54,55]. Over 30 years, many studies have focused on the descending pathways for pain modulation [42,47]. Many brain regions have been recognized to be implicated in the modulation of pain, such as rostral ventromedial medulla, periaqueductal gray, hypothalamus, amygdala, and frontal cortex [20]. These regions could directly influence the spinal cord excitability through inhibitory or facilitatory mechanisms [43,46]. However, it remains unclear whether the primary somatosensory cortex (SI), an important region for sensory processing, has a role in the descending modulation of pain.

Accumulated evidence suggests that the cortical sensory systems, including visual, auditory, and somatosensory cortices, have descending influence on sensory responses at both neuronal and behavioral levels. For example, Toda et al. found that the localized inhibition of visual cortex reduced visually evoked convergence eye movements in cats [53]. Suga et al. proposed that the auditory cortex adjusted and improved auditory signal processing in the subcortical auditory nuclei [52,59]. Using single-unit recording in awake, freely moving rats, Fanselow et al. showed that the descending signal from the primary somatosensory cortex (SI) triggered thalamic bursting during the whisker twitching behavior [17]. More recently, the influence of cortical modulation from SI cortex on the thalamic nociceptive relay neurons has been addressed by Monconduit et al. [37]. They found that the $GABA_A$-mediated depression of corticofugal outputs resulted in reduced noxious thermal-evoked responses of thalamic sensory neurons. In addition, our recent electrophysiological study in rats also indicated a substantial influence of SI on the processing of nociceptive inputs at the thalamic neuronal level [56]. Despite the important insights gained from these studies, little is known about how the cortical outputs modulate the nociceptive behaviors resulting from tissue injury or evoked by painful stimulation.

The study was designed to test whether primary somatosensory cortical output influences nociceptive behavior using rat models of acute pain induced by thermal or chemical (formalin) stimuli and chronic inflammatory pain induced by complete Freund's adjuvant (CFA). Since the majority of SI efferent neurons located in layer V and VI are primarily influenced by GABAergic interneurons [25,32,63], we used microinjections of a $GABA_A$ agonist and $GABA_A$ antagonist into SI to investigate the functional role of SI in descending pain modulation. Nociceptive behavior was assessed

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by measuring the paw withdrawal latency in the acute thermal and the chronic pain model and the time spent in licking the affected paw in the formalin test.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (250–300 g) obtained from the Laboratory Animal Center of the Academy of Military Medical Sciences had been used for this study. Animals were housed individually under a room temperature of 22 ± 1 °C with a 12 h light–dark cycle (light on at 07:00 AM), and had free access to food and water. The experimenter handled the animals daily to make them get used to the manipulation. Experimental procedures were approved by the Institutional Animal Care and Use Committee of Peking University and Chinese Academy of Sciences, and in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Surgery

Animals were deeply anaesthetised with sodium pentobarbital (50 mg/kg, i.p.). A stainless steel infusion guide cannula (30 gauge) was stereotaxically implanted into the SI cortex (1 mm posterior to the bregma, 2 mm lateral to the midline, 1.3 mm deep from the skull surface [41]). The tip of cannula was positioned in the internal granular layer. The guide cannula was fixed to the skull with three stainless steel screws and dental cement. Each cannula was kept patent with a sterile obturator until the time of drug administration. After receiving penicillin (16,000 U per rat, i.m.), rats were given 7 days to recover from the surgery.

2.3. Intracortical (IC) microinjection

The gamma-aminobutyric acid receptor ($GABA_A$) agonist muscimol (500 ng dissolved in 500 nl of normal saline, Tocris, Ellisville, MO) and antagonist bicuculline (500 ng dissolved in 500 nl of normal saline, Tocris, Ellisville, MO) were microinfused into layer VI of the SI cortex to alter the cortical activity. Control animals received the same volume of saline. The volume (500 nl) and concentration (1 ng/nl) of GABAergic drugs used in the present work were chosen according to the large number of published papers [4,16,21,24,26,28,37,39,40,51,57,58], in which intracerebral microinjection of muscimol or bicuculline was used to inactivate or disinhibit localized brain areas.

In this study, muscimol and bicuculline were injected at 30 min and 1 h, respectively, prior to the behavioral tests. Previous studies had suggested that it usually takes 30 min for the GABAergic drugs to take effect, and the effects persist for up to 6 h after microinjection [21,26,28,37]. In addition, it has been reported that intracortical administration of $GABA_A$ antagonist bicuculline may induce abnormal movements and postures in animals during a short period following injection [8]. In our preliminary test, we have noticed the abnormities and found that rats will recover to normal in about 30 min following bicuculline administration. Therefore, bicuculline was administrated at 1 h instead of 30 min before the behavioral tests.

The injection needle (a thin dental needle with 0.3 mm o.d.) was introduced through the guide cannula until its lower end was 1 mm below the guide cannula. The displacement of an air bubble inside the polyethylene catheter (PE-10) connecting the syringe needle to the intracerebral needle was used to monitor the microinjection. The solutions were slowly microinjected into the SI over a 1-min period. Then, the needle was held in place for an additional 1 min to maximize diffusion away from the tip of the needle.

2.4. Experimental protocol

The experimental paradigm used in this study was illustrated in Fig. 1. $GABA_A$ agonist muscimol or antagonist bicuculline was microinjected into SI cortex and led to cortical inactivation or activation. The effects of cortical descending modulation were explored on the acute thermal and chemical (formalin), and chronic inflammatory (CFA) pain models. Because of their respective features, the evaluation methods and experimental procedures of the three models differed. Both formalin- and CFA-induced pain were related to tissue injury and resulted in the development of persistent pain. The formalin-induced pain has been measured by the time spent licking the injected paw (spontaneous pain), whereas the CFA-induced pain has been evaluated by thermal-evoked hyperalgesia (evoked pain). In formalin model, we examined the drug effects over time, because the duration of formalin-induced behavior was in the time range of the drug effects (6 h). In CFA model, the drug effects were not measured over time and only tested on a given day (7th day after CFA), considering that the CFA-induced hyperalgesia lasted for up to 2–3 weeks which greatly exceeded the drug–effect range. For the thermal acute pain, it was evoked by brief cutaneous heat stimulation and was totally dependent on the presence of the noxious stimulus. Thus, the thermal pain-related behavior was not long lasting and time varying. The drug effects were only evaluated by measuring the mean paw withdrawal latency of the three single trials.

2.4.1. Formalin test

The formalin test was conducted 7 days following the cannula implantation. Testing sessions were carried out in a quiet room. The room temperature was maintained between 21 and 23 °C. Rats were first placed in a plastic test chamber (25 cm × 25 cm × 30 cm) for at least 10 min to accommodate to the environment. Then, they received IC microinjection of saline (control), muscimol, or bicuculline, followed by subcutaneous injection of 5% neutral formalin (50 μl) into the plantar surface of the hindpaw contralateral to

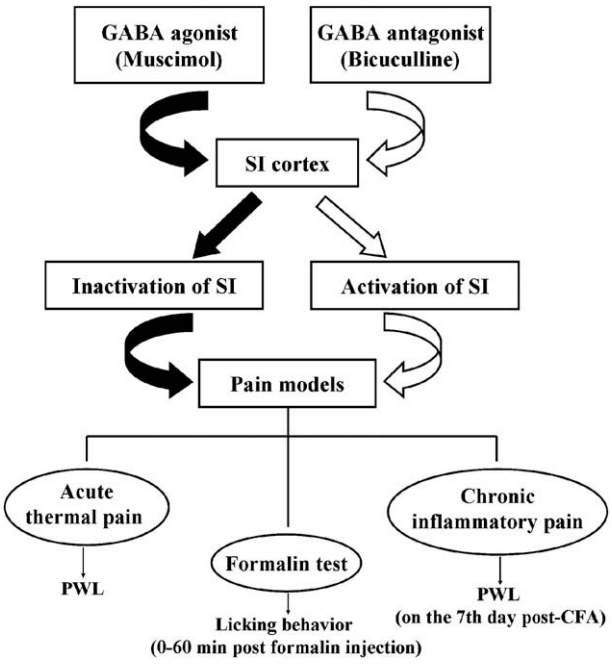

Fig. 1. Schematic diagram of the experimental protocol. GABAergic drugs were microinjected into SI cortex and led to the cortical inactivation (indicated by solid arrows) or activation (indicated by blank arrows). The effects of cortical descending modulation on the acute and chronic pain behaviors were explored.

the microinjection site. Rats were randomly allocated to receive one of the following treatments: (1) unilateral IC saline or muscimol 30 min before the formalin injection ($n = 8$); (2) unilateral IC saline, muscimol, or bicuculline 1 h before the formalin injection ($n = 8$); and (3) bilateral IC muscimol, bicuculline or saline 1 h before the formalin injection ($n = 7$). Then, rats were returned to the test chamber, and the nociceptive behaviors were videotape recorded throughout the following 60 min. Pain intensity was determined by measuring the time spent licking the affected paw every 5 min after injection.

2.4.2. Thermal acute pain

A radiant heat apparatus was employed to induce acute pain. Thermal thresholds of the rat hindpaws were measured. Each rat was placed in a plastic chamber on a glass floor under which the radiant heat apparatus (100-W projector lamp) located. A beam of light through a hole (4 mm diameter) in the apparatus was focused on the plantar surface of the left hindpaw. Paw withdrawal latency (PWL) was defined as the time length between the light onset and the paw lift, and was adjusted to around 10 s for the baseline level. A cutoff time of 22 s was applied to avoid tissue damage. Four trials with at least 5 min apart were conducted with each hindpaw. The last three trials were averaged to give a mean latency. Rats received one of the following treatments: (1) unilateral IC saline or muscimol 30 min before the thermal threshold test ($n = 8$); (2) unilateral IC saline, muscimol, or bicuculline 1 h before the thermal threshold test ($n = 10$); and (3) bilateral IC saline, muscimol, or bicuculline 1 h before the thermal threshold test ($n = 8$). The baseline thresholds were tested two days before the day when IC drugs were given.

2.4.3. Complete Freund's adjuvant induced chronic inflammatory hyperalgesia

Inflammatory pain was induced by intraplantar injection of 100 μl of Complete Freund's adjuvant (CFA, Sigma) into the hindpaw contralateral to the microinjection site. The thermal thresholds (paw withdrawal latency) of the inflamed and non-inflamed hindpaws were measured. The thermal stimulation tests were performed 1 day before (baseline), and 1, 3, 7, 12, 15, and 18 days after the CFA injection. At the 7th day following CFA injection, rats received IC microinjection and thermal threshold tests. The protocols were the same as those in the thermal acute pain experiment. Eight rats were used for each treatment. We performed intracortical administration on the day 7 post-CFA because a wealth of evidence has indicated that the nociceptive behaviors are relatively stable around day 7 after CFA injection [31,62].

2.5. Histology

After completion of the behavioral tests, animals were deeply anesthetised with pentobarbital sodium (60 mg/kg body weight, i.p.) and were perfused intracardially with 200 ml of sterile saline, followed by 400 ml of fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). The brain was removed and post-fixed in the same fixative for 16 h, and then cryoprotected in 0.1 M PB containing 20% sucrose until the tissue sank to the bottom of the container. The brains were sunk in 30% sucrose and were stored at 4 °C until sectioning. Frozen serial coronal sections (20 μm in thickness) were cut with a cryostat and mounted on gelatin-coated glass slides. The slides were stained with hematoxylin and eosin for verification of cannula placement in the SI. In the present study, the location of microinjection was in the layer VI, but the position of microinjection was 1 mm under cannula tip in order to avoid the drug circumfluence along the cannula. Accordingly, we verified whether the cannula tip was 1 mm above the layer VI of SI. Only data from rats in which accurate cannula placement was verified were included in the statistical analysis.

2.6. Statistical analysis

Data were presented as mean ± SEM Changes in nociceptive behaviors produced by IC drugs were analyzed by two-way (treatment × time) analysis of variance (ANOVA) followed by Newman–Keuls test. The three models were analyzed separately due to the different time points of data acquisition. In the formalin test, the cumulative time spent licking was analyzed by one-way ANOVA followed by Turkey's test or by Student's *t*-test for two groups. Differences were considered statistically significant at the $p < 0.05$ level.

3. Results

3.1. Formalin-induced acute inflammatory pain

3.1.1. Unilateral IC muscimol 30 min before formalin injection

In this experiment, formalin test began 30 min after IC saline or $GABA_A$ agonist muscimol. The time course of hindpaw licking in 5 min bins showed that both saline- and muscimol-administered rats displayed stereotypical biphasic behaviors (Fig. 2A). Compared with the saline control, muscimol administration significantly reduced the amount of nociceptive behaviors shown as a treatment × time interaction ($F_{(11,168)} = 2.4069$; $p = 0.0087$), notably at the time points of 40 and 45 min following formalin injection (15.79 ± 6.98 and 16.56 ± 6.82 vs. 60.03 ± 5.62 and 50.82 ± 7.28

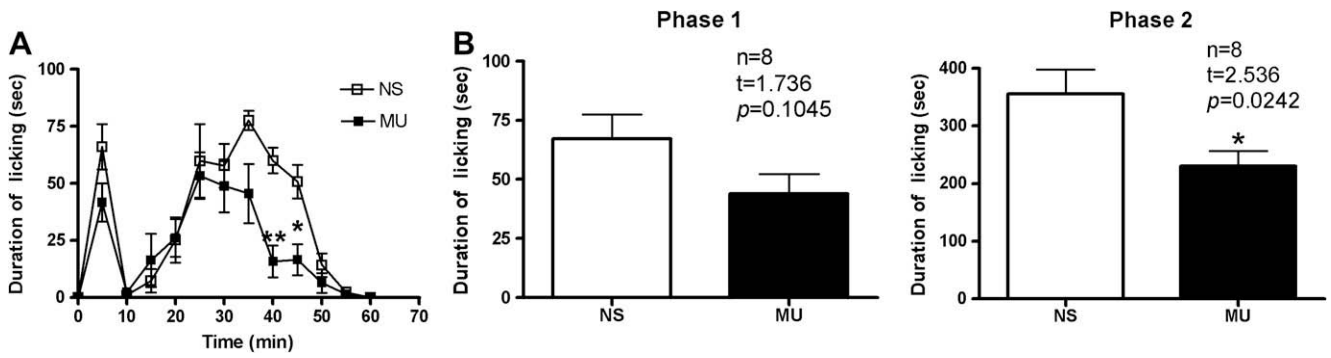

Fig. 2. Unilateral intracortical injection of muscimol 30 min before formalin injection selectively reduced phase 2 behaviors. Rats received a single injection of 5% formalin solution (50 μl) into hindpaw contralateral to the microinjection site. The nociceptive behavior was measured by the time spent in licking the injected paw, which varied as a function of time (A). Cumulative paw licking scores were not significantly different between muscimol and control group in phase 1 but in phase 2 (B). NS, saline; MU, muscimol. $^{*}p < 0.05$ and $^{**}p < 0.01$. $n = 8$.

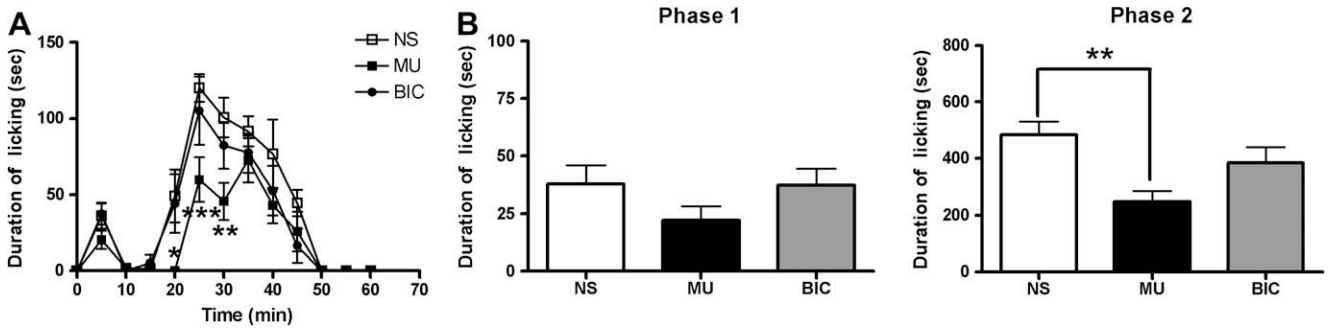

Fig. 3. Effects of unilateral intracortical muscimol or bicuculline 1 h before formalin injection. (A) Temporal function of pain behavior. (B) Cumulative licking score in phases 1 and 2. Muscimol significantly attenuated the second phase behaviors; in contrast, bicuculline failed to show any effect on any of the two phases. NS, saline; MU, muscimol; BIC, bicuculline. $^{*}p < 0.05$ and $^{**}p < 0.01$. $n = 8$.

for muscimol and control group, respectively; Newman–Keuls post hoc test, $p = 0.0039$ and $p = 0.0302$, respectively). The data of cumulative time spent licking in phase 1 (0–10 min) and phase 2 (11–60 min) clearly showed the decrease, especially in the second phase behavior by muscimol microinjection (for phase 1, 67.20 ± 10.38, and 43.97 ± 8.44, respectively, $t(14) = 1.736$, $p = 0.1045$; for phase 2, 355.4 ± 42.23 and 230.5 ± 25.69, respectively, $t(14) = 2.526$, $p = 0.0242$, see Fig. 2B). These results indicated that the inhibition of SI activity could attenuate formalin-induced pain, suggesting a facilitatory role of the SI in the descending control of acute inflammatory pain.

3.1.2. Unilateral IC muscimol or bicuculline 1 h before formalin injection

For the next experiment, saline, muscimol or $GABA_A$ antagonist bicuculline was unilaterally microinjected into SI cortex 1 h before the formalin injection. As was the case for microinjection 30 min pre-formalin, a significant decrease in the second phase behaviors was observed by muscimol administration (two-way ANOVA, treatment × time interaction, $F_{(18,140)} = 2.5107$, $p = 0.0112$, Fig. 3A), specifically at the time points of 20, 25, and 30 min following formalin injection (0.00 ± 0.00, 60.03 ± 14.47 and 45.79 ± 12.18 vs. 49.28 ± 17.17, 120.2 ± 9.21 and 100.7 ± 13.13 for muscimol and control group, respectively; Newman–Keuls post hoc test, $p = 0.0334$, 0.0005, and 0.0029, respectively). By contrast, there was no significant effect of bicuculline on the two phases (interaction, $F_{(18,140)} = 0.4529$; $p = 0.9032$; treatment, $F_{(1,140)} = 1.9473$; $p = 0.1846$). The cumulative data shown in Fig. 3B revealed marked decrease in the second phase by muscimol (247.5 ± 38.59) but not bicuculline (383.8 ± 55.57) administration (one-way ANOVA, $F_{(2,21)} = 6.294$, $p = 0.0072$), when compared to the saline control (484.3 ± 46.40, Turkey's post hoc analysis, $p < 0.01$).

3.1.3. Bilateral IC muscimol or bicuculline 1 h before formalin injection

To determine the contribution of bilateral SI to the descending control of pain, simultaneous bilateral administration into SI was performed. As expected, muscimol administration produced profound anti-nociceptive effects, with both phase 1 and phase 2 behaviors remarkably reduced (two-way ANOVA, treatment effect, $F_{(1,120)} = 18.9385$; $p = 0.0009$, Fig. 4A), especially at the time points of 5 and 25 min following formalin injection (16.16 ± 6.83 and 32.41 ± 14.98 vs. 80.15 ± 12.60 and 93.00 ± 14.38 for muscimol and control group, respectively; Newman–Keuls post hoc test, $p = 0.0084$ and 0.0142, respectively). One-way ANOVA analysis of the cumulative data showed that both the first phase and the second phase were greatly inhibited (for phase 1, $F_{(2,18)} = 9.910$, $p = 0.0013$, post hoc analysis between muscimol and saline, $p < 0.01$; for phase 2, $F_{(2,18)} = 6.302$, $p = 0.0084$, post hoc comparison between muscimol and saline, $p < 0.01$, see Fig. 4B). Consistent with the preceding result, there was no effect of bicuculline on the two phase behaviors.

3.2. Thermal-induced acute pain

We also investigated the effects of IC saline, muscimol, or bicuculline on the acute thermal nociceptive thresholds measured with noxious radiant heat in normal rats. It was found that unilateral or bilateral IC muscimol significantly increased the paw withdrawal latency (PWL) as compared to the saline control (unilateral IC with 30 min latency, $p = 0.0185$, Newman–Keuls test following the two-way ANOVA, Fig. 5A; unilateral IC with 1 h latency, $p = 0.0224$, Fig. 5B; bilateral IC with 1 h test, $p = 0.0097$, Fig. 5C). In contrast to the analgesic effect of muscimol, bicuculline did not have any effect on the withdrawal behaviors induced by noxious heat stimulus, regardless of unilateral (Fig. 5B) or bilateral (Fig. 5C)

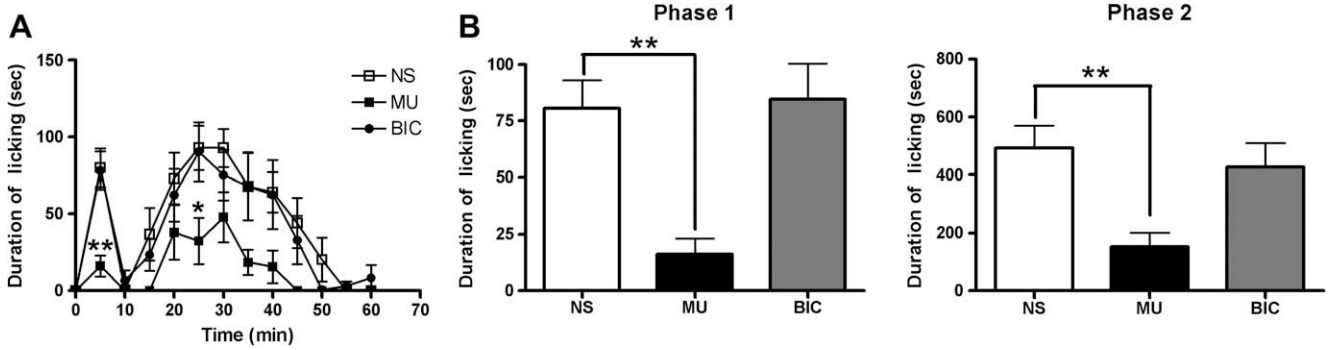

Fig. 4. Effects of bilateral intracortical muscimol or bicuculline 1 h before formalin injection. (A) Temporal function of pain behavior. (B) Cumulative licking score in phases 1 and 2. Muscimol profoundly inhibited the nociceptive responses in the first and second phases; by contrast, no significant effect was seen following bicuculline administration. NS, saline; MU, muscimol; BIC, bicuculline. $^{*}p < 0.05$ and $^{**}p < 0.01$. $n = 7$.

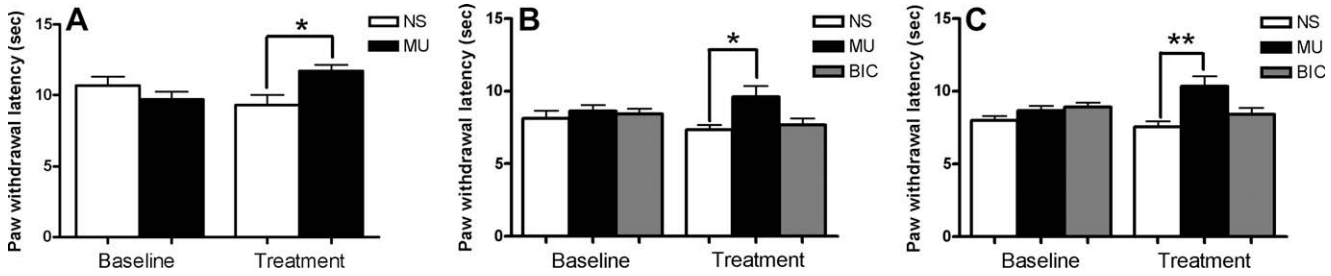

Fig. 5. Effects of intracortical muscimol or bicuculline on the nociceptive thermal thresholds in normal rats. Unilateral (A and B, 30 min and 1 h before testing, respectively) or bilateral (C, 1 h before test) application of muscimol significantly increased the PWL evoked by noxious heat stimulation. In contrast, neither unilateral nor bilateral IC bicuculline affected the withdrawal thresholds. NS, saline; MU, muscimol; BIC, bicuculline. $^{*}p < 0.05$ and $^{**}p < 0.01$. $n = 8$–10.

administration. These data demonstrated that inhibition of cortical activity could increase the thermal acute pain threshold. This result, together with that obtained in the formalin test, confirmed the facilitatory effect of the SI descending control on the acute pain.

3.3. CFA-induced chronic inflammatory hyperalgesia

In this experiment, we first evaluated the validity and reliability of the animal model of CFA-induced inflammatory pain. Fig. 6A shows the time course of thermal hyperalgesia related to CFA injection. Before the CFA injection, there was no significant difference in the PWL among all groups of rats and between left and right hindpaws. Following the injection of CFA, there was a significant decrease in PWL of the injected paw compared to the pre-CFA baseline and the non-injected paw (lateral effect: $F_{(1,98)} = 24.9691$; $p = 0.0002$, time effect: $F_{(6.98)} = 4.5494$; $p = 0.0005$). The thermal hyperalgesia started at day 1 post-CFA and persisted through day 18.

On the day 7 post-CFA, saline, muscimol or bicuculline was microinjected into SI cortex. Then, the rats received noxious heat stimulation and PWLs were measured, as in the thermal acute pain experiment, to examine the effects of cortical descending modulation on the CFA-induced chronic inflammatory pain. In the experiment of

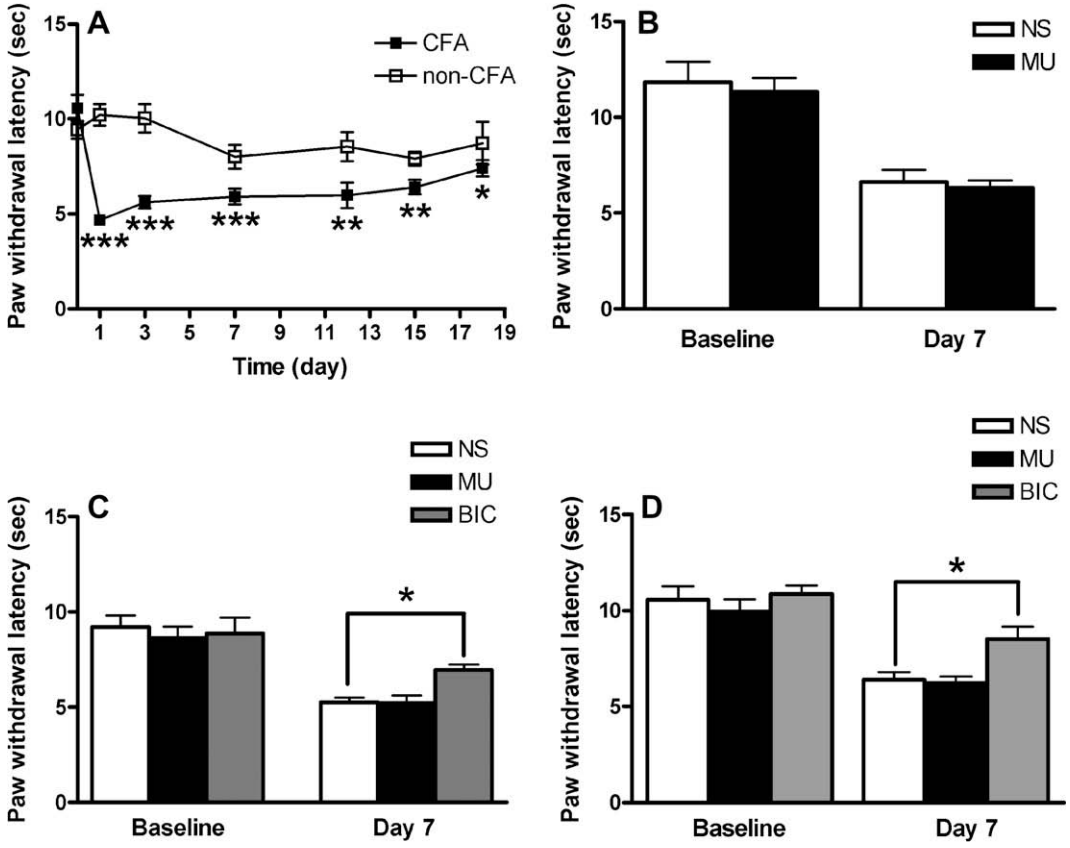

Fig. 6. Effects of intracortical muscimol or bicuculline on CFA-induced chronic inflammatory pain. Microinjection contralateral to the CFA injection was performed on the day 7 post-CFA. (A) Time course of CFA-induced thermal hyperalgesia. Noxious radiant heat was delivered to the CFA and non-CFA injected paws. Before the CFA injection, there was no significant difference in PWL between left and right hindpaws. Following the injection of CFA, there was a significant decrease in PWL of the injected paw compared to the pre-CFA baseline and the non-injected paw, which started from day 1 post-CFA and persisted until day 18. Unilateral (B and C, 30 min and 1 h before testing) or bilateral (D, 1 h before testing) IC muscimol did not alter the nociceptive thermal thresholds. In contrast, both unilateral and bilateral IC bicuculline significantly increased PWL of the inflamed paw. NS, saline; MU, muscimol; BIC, bicuculline. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the baseline level. Baseline refers to the pre-CFA threshold level without IC drugs. $n = 8$.

microinjection of muscimol 30 min (Fig. 6B) or 1 h (Fig. 6C and D) before thermal stimulation, no significant difference was found between muscimol and saline groups in the PWL of the inflamed paws. Interestingly, bicuculline produced significant increase in PWL of the CFA-injected paws for both unilateral (Fig. 6C) and bilateral (Fig. 6D) administrations (Newman–Keuls post hoc test following two-way ANOVA, $p = 0.0361$ and $p = 0.0187$, respectively). The elevation of nociceptive thresholds produced by the activity of SI efferent neurons suggested that the SI outputs were inhibitory in the chronic inflammatory pain state, contrary to that in the acute pain states.

3.4. Histology

Histological observation from serial sections demonstrated that the cannula tips of most of the rats were located in the internal granular layer of SI (layer IV), as shown in Fig. 7. Because the end of injection needle (Fig. 7C, dashed arrow) was 1 mm below the cannula indentation (Fig. 7C, solid arrow), the injection site should be in the sixth layer of the SI. In the present study, a total of 220 rats were used at the beginning of the experiment. Histological results indicated that the cannula implantations of nearly 30 rats were missed. Therefore, the related data were not included in the final analysis.

4. Discussion

The major finding of this study was that the formalin- or noxious thermal-induced acute pain could be attenuated by the $GABA_A$-mediated inhibition of the SI cortex; in contrast, the CFA-induced chronic pain could be relieved by the disinhibition of SI. These results represent direct evidence that the corticofugal outputs from SI are capable of facilitating acute pain but inhibiting chronic pain.

It has long been recognized that the role of SI cortex in the pain processing primarily involves the sensory-discriminative aspect such as stimulus location, duration, and property [5,12,13,29]. Few studies have focused on the cortical feedback modulation of the nociceptive transmission. Although it has been proposed that there are substantial projections from SI to thalamic relay nuclei [27,33], and the corticofugal outputs could influence the response properties of ventroposterolateral (VPL) thalamic neurons to noxious inputs [45], no study has examined the descending effects of SI on the pain-related behaviors resulting from tissue injury or evoked by noxious stimulation. The present study provided the first evidence for the influence of corticofugal outputs on the nociceptive behaviors using rat models of noxious thermal-induced acute pain, formalin-induced acute and CFA-evoked chronic inflammatory pain.

4.1. The facilitatory effects of SI in acute pain states

In our study, intracortical administration of $GABA_A$ agonist muscimol significantly reduced the first and second phase behaviors in the formalin tests and elevated the nociceptive thresholds in the thermal stimulus-elicited acute pain. This was in accordance with the previous electrophysiological study, in which $GABA_A$-mediated depression of SI depressed noxious-evoked responses of VPL thalamic neurons [37]. Similar effects have also been found by other studies. Yuan et al. have reported that inactivation of SI decreases the thalamic neural responses to acute cutaneous electrical stimulation [60,61]. Zhuo has shown that the activation of the anterior cingulate cortex (ACC) with high-frequency tetanic electrical stimulation shortened the PWL to acute thermal stimulation [64]. Based on the present results and the evidence described above, we postulate that the reduction in the pain-related behaviors by inhibition of SI may be associated with the suppression of thalamic nociceptive transmission. This needs to be further investigated.

On the other hand, the observation that excitation of SI by bicuculline did not produce apparent changes was not in agreement with Monconduit et al., who found that both glutamatergic activation and $GABA_A$-antagonist-mediated disinhibition of SI enhanced the noxious-evoked responses of VPL neurons. This inconsistency may be attributable in part to the difference in the methodological approaches between the two studies. In the electrophysiological study by Monconduit et al., anesthesia was employed in rats. It is possible that the somatosensory neurons have not been totally excited by noxious stimulation under the condition of anesthesia. As a result, the cortical activity has been recovered to some extent by the pharmacological excitation of the cortical neurons. By contrast, awake freely moving rats were used in our study for the nociceptive behavioral tests. It is likely that the SI cortex has already been too excited by the peripheral noxious stimuli to be further activated pharmacologically. An alternative explanation for the inconsistent results could be that the GABAergic activity within SI cortex was relatively weak in the acute pain state. As a consequence, the pro-GABAergic drug would produce a significant increase in the nociceptive threshold, whereas the anti-GABAergic drug would have little effect on it. However, further studies are needed to understand the inconsistent findings regarding the effect of a $GABA_A$ antagonist injected into SI activity onto nociceptive behavior.

4.2. The inhibitory effects of SI in chronic pain state

Microinjection of bicuculline remarkably increased the PWL of the inflamed paws, indicating that the somatosensory cortex tends to suppress the nociceptive processing in the chronic pain state.

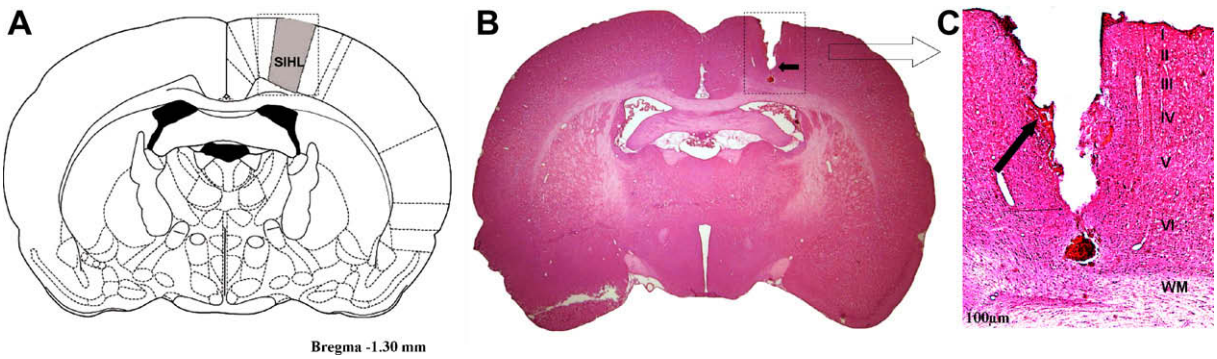

Fig. 7. The location of cannula implantation within the SI cortex. (A) A coronal section modified from the atlas of Paxinos and Watson (bregma −1.30 mm). The gray shaded area marks the SI hindpaw representation for cannula implantation. (B) Histochemical staining of the brain section with hematoxylin and eosin. The cannula indentation is indicated by the arrow. (C) Light microscopic examination with higher magnification demonstrates the microinjection site in the brain section in (B). The solid arrowhead indicates the position of cannula tip. The dashed arrowhead indicates the injection site. Roman numerals (I–VI) mark cortical layers. Scale bar = 100 μm; WM, white matter.

Such a result was similar to those found in human-imaging studies, in which severe chronic pain was associated with the loss of somatosensory neurons [3,49]. Although it is unclear whether the loss of neurons was limited to excitatory neurons in these studies, chronic pain may be explained as a disturbance in the balance of excitation and inhibition of the brain.

Unexpectedly, we did not find a significant influence by muscimol administration on the CFA-inflamed paws. It is conceivable that the degree of pain sensations in the inflamed paw was severe enough, thus it was difficult to detect more facilitatory effects caused by muscimol. Another possible interpretation was that the GABAergic transmission within SI may be enhanced to the greatest extent under the condition of chronic pain. For this reason, $GABA_A$ antagonist could give rise to significant pain relief, whereas $GABA_A$ agonist failed to produce stronger effects than saline. However, these possible explanations have to be tested in the future studies.

The recent studies have shown that $GABA_A$ receptor activation can lead to depolarization of cortical neurons [22,34]. The accumulation of intracellular Cl^- ($[Cl^-]_i$) may be responsible for this effect. Given this, it is possible that the decrease in CFA-induced pain by bicuculline application might be due to the block of excitatory $GABA_A$-receptor-mediated actions on cortical pyramidal cells. However, only several pathological conditions, for example, epilepsy and local ischemia, have been documented in which increased $[Cl^-]_i$ in cortical neurons was observed [7,50]. Although elevated $[Cl^-]_i$ was also found in the dorsal horn neurons after peripheral nerve injury or inflammatory hyperalgesia [9,19], no study has yet demonstrated a GABA-induced excitation of cortical neurons in the chronic pain states. Thus, it is not clear whether the peripheral inflammatory damage is sufficient to switch the GABAergic synapses from inhibitory to excitatory.

4.3. Implications of the opposite effects for acute and chronic pain

From the perspective of biological adaptation, it is possible that the corticofugal influence of SI cortex on the acute and chronic pain is different. Under normal physiological conditions, noxious stimulus activates not only ascending nociceptive pathways but also descending endogenous modulatory systems [2,10,15,23]. Activation of the descending facilitatory systems results in faster escaping responses, thus to avoid further tissue injury [38]. We believe that the cortical outputs facilitating the escaping responses may serve a protective function in the acute pain condition.

By contrast, when the tissue damage is inescapable, i.e., in the state of chronic pain, the SI descending inhibitory system may be more active than the facilitatory system, as shown in this study. Although many studies have indicated that the central network mediating chronic pain predominantly involves facilitatory mechanism in the spinothalamic pathways [18,20,44,65], Crick and Koch have pointed out that the corticothalamic circuits never form strong directed loops, because too strong excitatory loop would throw the cortex into uncontrolled oscillations, as in epilepsy [11]. Thus, there is likely to be inhibitory influence from SI on the abnormal nociceptive transmission in chronic pain state.

4.4. Methodological considerations

Intraplantar injection of formalin is an ideal model for studying pain as it consists of two transient and stereotyped phases of pain behavior. In general, the first phase is considered to be an acute pain state and is thought to result from direct activation of nociceptors; the second phase is a delayed inflammatory state, which involves not only prolonged activity of nociceptors but also a first phase-induced central sensitization of pain transmission circuits [1,14]. Although there is a controversy over whether the formalin-induced behaviors reflect acute or chronic pain, many pharmacological studies employed the formalin test as an acute inflammatory pain model in contrast to the CFA-induced chronic pain [6,31]. In our study, the descending influence by SI in the formalin test was consistent with that in thermal acute pain, but contrary to that of CFA-induced pain. This suggested that the formalin-induced pain most likely represented the status of acute pain.

A limitation of our study is that behavioral arousal may be an influencing factor given the fact that the behavioral data were collected in fully awake animals. It has been reported that the degree of cortical arousal can influence behavioral reactivity to external stimuli in a bi-modal manner as described by the inverted U-shaped relationship [30,35]. Thus, it is possible that muscimol shifted animals into lower arousal state rendering them less reactive in the acute pain models, whereas CFA induced heightened arousal rendering the animals also less reactive if further "aroused" by bicuculline treatment. The possible effect of cortical arousal needs further study.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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